CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

W. F. GEDDES, Editor-in-Chief
R. J. TARLETON, Managing Editor
EUNICE R. BROWN, Editorial Asst.
HARLEY L. WARD, Advertising Mgr.
University Farm, St. Paul 1, Minn.

CONTENTS

Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1934. Published at 500 So. 5th St., Minneapolis, Minn. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

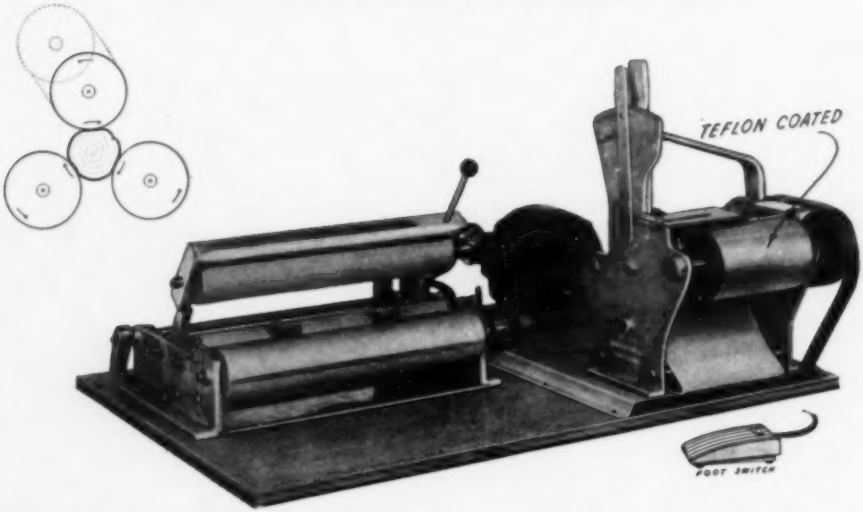
Vol. 33 May, 1956 No. 3

SOMETHING NEW for your Lab.!

The National

Teflon Coated SHEETING ROLL

as shown with Three-roll-type Moulder



"THREE-ROLL-TYPE" MOULDER

for all size loaves

Price—Moulder Only—$110.00

Includes flexible coupling which drives the unit from speed reducer of the sheeting roll. Also gauge pins for both "pups" and larger loaves.

Test Bake Moulding by "THREE-ROLL-TYPE" Moulder gives a mechanized action which is

(1) Gentle
(2) Effective
(3) Ends of doughs not abused
(4) Operation is visible

1 lb. SIZE (6" Width) TEFLON COATED SHEETING ROLL

Adjustable to sheet "Pups"

PRICE — Sheeter only, Teflon Coated—$240

Includes motor, speed reducer with power take-off for moulder and foot switch.

"PUP" SIZE (3" Width) SHEETING ROLL

with same equipment

$200.00

IF THE SHEETER AND MOULDER ARE ORDERED AT THE SAME TIME THEY ARE MOUNTED ON A COMMON BASE AS SHOWN IN ILLUSTRATION AT NO EXTRA COST.

CEREAL CHEMISTS' EQUIPMENT MANUFACTURER

NATIONAL MFG. COMPANY

LINCOLN, NEBRASKA

INSIDE SCIENCE

The Vital Story of Vitamin B₁

(Thiamine)

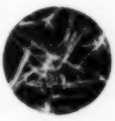
by Science Writer

History. The discovery of vitamin B_1 resulted from research into the cause of beriberi. Almost 50 years passed between Eijkman's discovery of the relationship of the disease to diet and the famous work of Jansen and Donath who first isolated the crystalline vitamin from rice bran.

Within ten years of that first isolation the vitamin's chemical structure was determined and it was successfully synthesized.

Eijkman's work resulted in the development of a theory that beriberi was caused by a lack of some factor in the diet and not by a toxin or infectious agent. This idea was not readily accepted until the growth of dietary knowledge proved it correct.

Isolation and Synthesis. In 1926 Profs. Jansen and Donath accomplished the isolation of crystalline vitamin B_1 from rice bran. In 1931 Windaus and coworkers successfully isolated pure vitamin B_1 and established its empirical formula. In 1936 R. R. Williams, and independently R. Grewe, explained the vitamin's chemical structure. That year, R. R. Williams and J. K. Cline accomplished the synthesis of thiamine which is in wide use today. Andersag and Westphal also synthesized the vitamin in 1936. Another synthesis was described by Bergel and Todd in 1937.



Photomicrograph of B_1 crystals

Chemical and Physical Properties. Thiamine hydrochloride is white, water soluble, with a nut-like, salty taste and yeast-like odor. Its empirical formula is: $C_{12}H_{17}ClN_4OS \cdot HCl$. Thiamine produced by synthesis is identical chemically and in biological activity with that obtained in pure form from nature.

Deficiencies. A deficiency of thiamine is characterized by these symptoms: depression, irritability, fearfulness, lack of initiative and interest, loss of appetite. Symptoms vary since in usual practice deficiencies of other water-soluble vitamins occur. Medical treatment is simple: a sufficient amount of thiamine is administered to relieve symptoms quickly and the physician provides for a continuing adequate intake.

Beriberi victim

A severe deficiency of thiamine leads to beriberi, a serious and sometimes fatal disease. While beriberi is almost a medical curiosity in the United States, it is common in countries in which polished white rice is a staple of the diet.

Human Nutrition Requirements. Thiamine is one of the nutritive elements the human body needs daily and does not store in quantity. The minimum daily requirements established by the U. S. Food and Drug Administration for the prevention of symptoms of thiamine deficiency disease are:

Adults ....1.00 mg.	Children (1-5 incl.)..0.50 mg.
Infants ....0.25 mg.	Children (6-11 incl.).0.75 mg.

The Food and Nutrition Board of the National Research Council recommends the following dietary intake of thiamine for healthy persons in the U. S. A.

Recommended Daily Intake in Milligrams

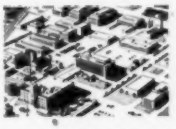
Age	*Men*	*Women*
25	1.6	1.2
45	1.5	1.1
65	1.3	1.0
Pregnant (3rd trimester)		1.5
Lactating		1.5

The Council recommendations for infants and children vary below and above these figures, based on age and sex. Various illnesses and stress situations can exhaust vital reserves of thiamine. So, for the physician, vitamin B_1 is prepared in various dosage forms and potencies for therapeutic and prophylactic use.

How do human beings receive thiamine? It is widely distributed in foods of animal and vegetable origin, particularly cereal grains and dry legumes. Because of public demand for refined products which millers must meet for obvious economic reasons, a loss of thiamine and other factors occurs during processing. The thiamine loss is overcome through the use of *enrichment* in cereal grain products for which Federal Standards exist, or in other foods such as breakfast cereals, by *fortification* or *restoration*. When enriching, fortifying or restoring, the food processor adds the necessary amount of pure thiamine (and other vitamins and minerals) to the food so that the finished product meets Federal, state and territorial requirements or contributes to the consumer an amount of the vitamin which dietary experts believe significantly useful.

Thiamine is extensively used for the enrichment of cereal grain foods such as white flour, white bread and rolls, macaroni products, farina, corn grits and meal, milled white rice. The story of these uses is delightfully told in a separate brochure which is available on request for reference or educational purposes.

Production. Huge production facilities at the Hoffmann-La Roche plant in Nutley, New Jersey, deliver highest quality thiamine *by the tons*. Roche manufactures thiamine hydrochloride and thiamine mononitrate. These fine products, which equal or exceed U.S.P. specifications, are ideal for use by pharmaceutical makers and food processors. Years of experience in research and manufacture have made Roche the leader in vitamins.



This article is published in the interests of pharmaceutical manufacturers, and of food processors who make their good foods better with essential, health-giving vitamin B_1. Reprints of this and others in the series are available on request. Write the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

what's her **VERDICT?**

Blue Ribbons or

Every day thousands of housewives and their families sit in judgment on your product. Their verdict . . . blue ribbon or brickbats—is reflected in your sales charts.

Each housewife buys your self-rising flour or corn meal because in the proving grounds of her own kitchen, she's learned to *depend* on your product. Years of success have demonstrated its reliability.

Tamper with your successful formula and she's quickly aware of it. She'll notice even the most subtle changes. When this happens her loyalty wavers.

There is one sure way of keeping her good will and continuing loyality. Buy the ingredients you know are best . . . like Victor's dependable V-90.

For 17 years virtually all the self-rising flour and self-rising corn meal made in America has been leavened with V-90. It is a proved, successful, reliable leavening agent.

Don't risk the reputation of your product. Specify V-90 and be sure.

THE ORIGINAL PHOSPHATE WITH A RAINCOAT*

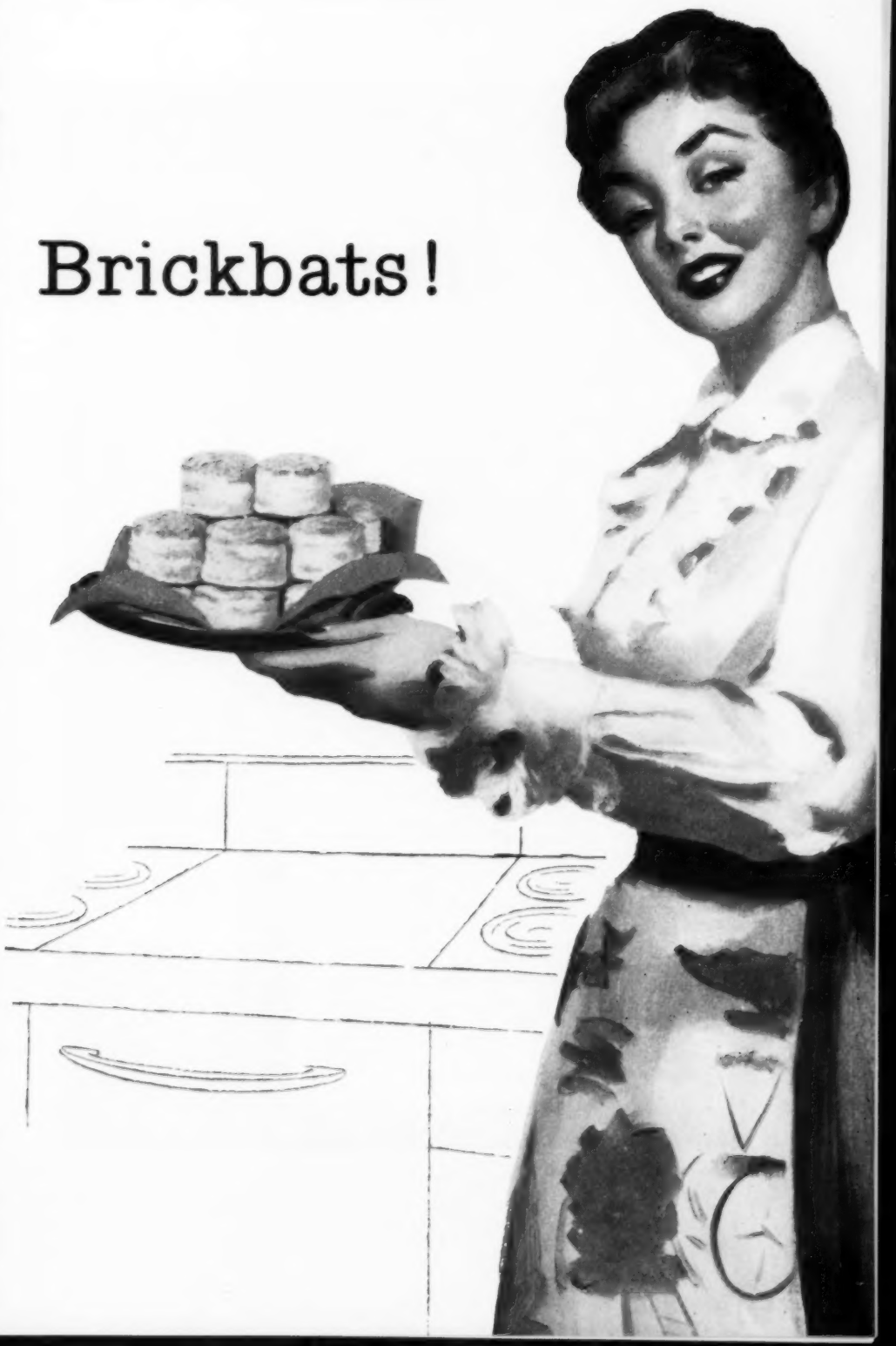
V-90®

*Coated Anhydrous Monocalcium Phosphate

VICTOR CHEMICAL WORKS

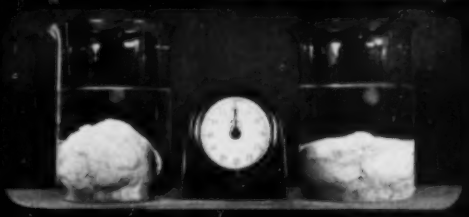
155 N. WACKER DRIVE CHICAGO 6, ILLINOIS

Brickbats!

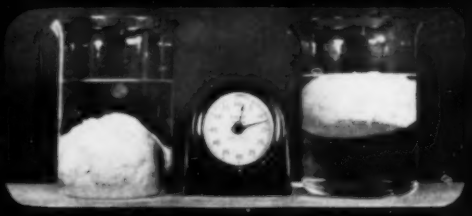


Proof PY-RAN* Leavens in the Oven!

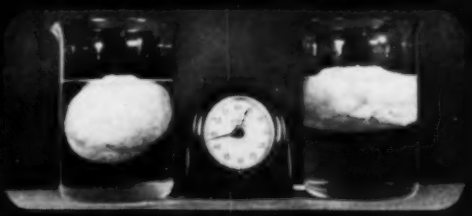
Freshly mixed doughballs made with self-rising flour rest in liquid. Dough at left leavened with PY-RAN; another calcium phosphate in dough at right.



2 minutes, 12 seconds: PY-RAN dough remains at bottom. The other calcium phosphate has evolved so much CO_2 that doughball floats to top.



3 minutes, 44 seconds: PY-RAN dough finally begins rise, proof of slow controlled gas release during critical pre-oven period.



PY-RAN (anhydrous monocalcium phosphate) not only holds its leavening action for the oven, it gives longer shelf life to prepared mixes and self-rising flour. A special moisture-absorbent coating protects PY-RAN from reaction during storage. PY-RAN performs well with other leavening acids, gives superb texture, volume, crust and crumb color, reduces tunneling.

You will also like SAPP-40 for machine doughnut mixes, SAPP-28, a slow-action baking acid, and HT* Phosphate (MCP monohydrate). Ask the local Monsanto representative for the new book "Monsanto Phosphate Leavening Agents," or write: Monsanto Chemical Company, Inorganic Chemicals Division, 710 North Twelfth Boulevard, St. Louis 1, Missouri.

*Reg. U. S. Pat. Off.

MONSANTO

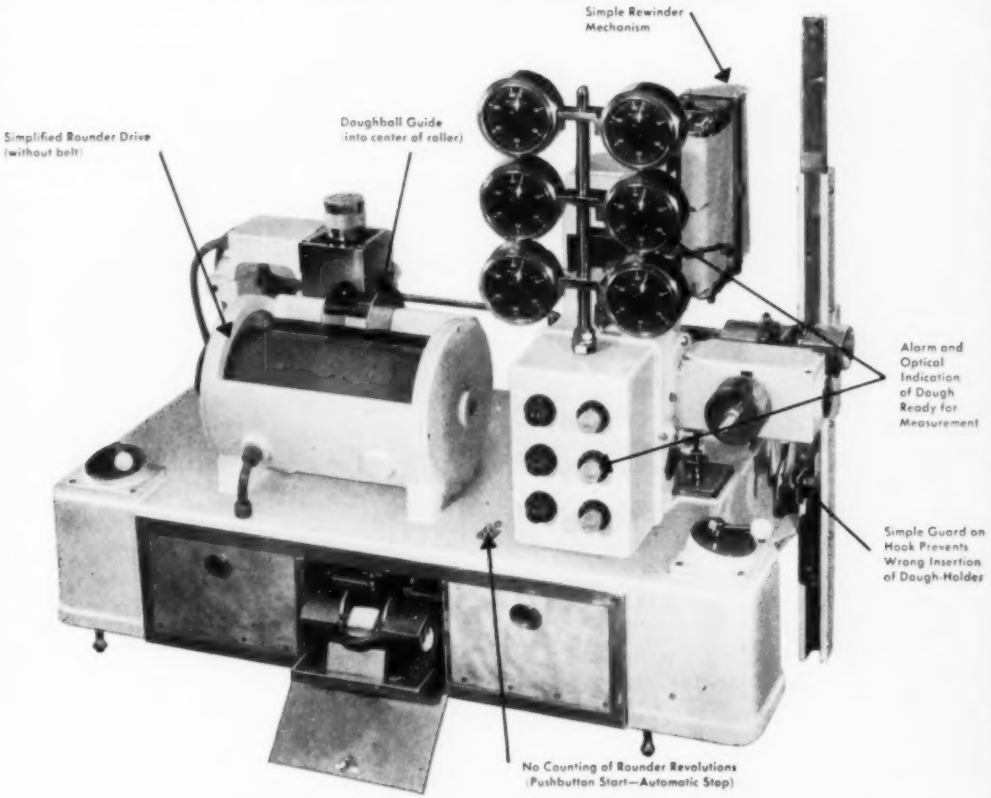
SERVING INDUSTRY... WHICH SERVES MANKIND

WORLD'S LARGEST PRODUCER OF ELEMENTAL PHOSPHORUS

ORIGINAL BRABENDER EXTENSIGRAF

(RECORDING EXTENSIMETER)

1956 MODEL NOW AVAILABLE



PERFORMANCE IMPROVED
PRICES REDUCED

MIAG NORTHAMERICA, INC.

1616 South 8th St., Minneapolis 4, Minnesota

Telephone: FEderal 9-5713

Exclusively franchised Sales and Service Agents for genuine C. W. Brabender invented instruments.

U. S. Factory

C. W. BRABENDER Instruments, Inc.

P. O. Box 106 — South Hackensack, N.J.

CEREAL CHEMISTRY

VOL. 33 MAY, 1956 NO. 3

THE EFFECTS ON FLOUR DOUGH AND BREAD QUALITY OF MOLDS GROWN IN WHEAT AND THOSE ADDED TO FLOUR IN THE FORM OF SPECIFIC CULTURES[1]

Y. POMERANZ,[2] P. HALTON,[3] AND F. G. PEERS[4]

ABSTRACT

The increase in mold population resulting from storing wheat at high levels of moisture and temperature resulted in a fall in test weight and fat content and an increase in fat acidity. The thiamine and, to a smaller degree, the nicotinic acid decreased, whereas the riboflavin increased.

On milling a greater yield of flour was obtained, owing at least in part to greater bran fracture. The flour had poorer color, increased ash, lower fat content, and greater fat acidity.

In the early stages of storage some improvement in baking quality was found, but later the water absorption, dough characteristics, and bread quality showed marked deterioration. Small additions of moldy flour were found to improve the baking quality of sound flour, but larger additions caused deterioration.

Cultures of *Aspergillus flavus* and *A. ochraceus*, when added to flour, caused marked deterioration in water absorption, dough properties, and bread quality. *A. candidus* and *A. amstelodami* had less deleterious effects and *Penicillium chrysogenum* had a negligible effect. *A. niger* caused a marked drop in water absorption and in some respects adversely affected dough properties; bread quality was, however, improved. *Alternaria tenuis* caused a small drop in water absorption but showed an improvement in the stretching characteristics of dough similar to that produced by oxidation. Loaf volume was, however, little affected, although crumb quality was improved. Some of the effects were produced by substances which were soluble in weak calcium chloride solution and were heat-labile.

The effect of molds on dough and bread quality can be partly explained by a balance between improvement due to increased amylase activity and deterioration due to marked proteolytic activity, but it seems possible that other active substances may be elaborated during the growth of molds on bran mash.

When wheat is stored at high moisture and suitable temperature levels the growth of molds is favored, and Swanson (22) found that this resulted in decreased viability, decreased test weight, increased fat acidity, increased total sugars, and deterioration in baking quality.

[1] Manuscript received February 10, 1955.

[2] Holder of F.A.O. Fellowship at the Cereals Research Station, St. Albans, England; present address, Ministry of Food and Industry, Food Testing Laboratory, Haifa, Israel.

[3] Research Association of British Flour-Millers, Cereals Research Station, St. Albans.

[4] Formerly of the Cereals Research Station, St. Albans; present address, Veterinary Research Laboratory, Vom, Northern Nigeria.

Swanson (23) also found an increase in flour yield on milling, the flour having high ash, decreased water absorption, and inferior baking quality. Leavitt and LeClerc (18) found that the total sugar content tended to increase. Zeleny and Coleman (25) showed that the fatty acids increased significantly during the early stages of spoilage, but that acid phosphates increased only after moderate deterioration and amino acids only in badly damaged grain. Bayfield and O'Donnell (3) showed that the thiamine content of wheat can decrease markedly under unfavorable storage conditions, but little information is available concerning changes in the other B vitamins.

Sorger-Domenigg *et al.* (21), working with mold-inoculated wheat kept at various moisture contents up to 21% for periods up to 15 days, found that after drying to 14% moisture germ damage and fat acidity continued to increase despite a fall in the mold counts. Milling quality was adversely affected by preliminary storage at 21% moisture, and these highly infested samples gave a higher yield of flour having inferior baking quality. This last continued to decline during subsequent storage at 14% moisture.

In regard to the species of molds found in wheat, Gilman and Semeniuk (12) quoted *Aspergillus* and *Penicillium* as being the most predominant fungi on various types of grain. Milner, Christensen, and Geddes (19) found that *Alternaria* constitutes about 90% of the total mold population of a sound wheat, whereas in "sick" wheat approximately 80% of the spores were of *Aspergillus glaucus* or *Penicillium* spp.

Greer and Hutchinson[5] suggested that different molds grown on damp wheat have varying influences on baking quality of milled flours. Thomas (24) studied the role of fungi in sick wheat and found that, of species grown on sterile bran, the greatest toxicity to germination was shown by culture filtrates of *A. flavus, A. niger,* and *Penicillium* spp. Bottomley *et al.* (4) showed that the growth of *A. glaucus* on damp wheat caused a rapid loss of nonreducing sugars, whereas *A. candidus, Penicillum* spp., and *Fusarium* spp. showed marked increases in fat acidity. Milner, Christensen, and Geddes (19), using specially grown, surface-sterilized, sound wheat inoculated with pure cultures of various fungi, found a lowering of viability by *Aspergillus* spp. and suggested that the deleterious effects of molds are due mainly to their lipolytic activity.

The objects of the present investigation were, first, to make a preliminary study of the effects produced by mold growth on damp

[5] Greer, E. N., and Hutchinson, J. B. Private communication.

wheat, and then to examine the effects on dough properties and baking quality produced by pure cultures of certain fungi.

Materials and Methods

Wheat Used. Previous experience in these laboratories had shown that flour milled from moldy wheat gave doughs which became very soft and extensible during fermentation. To allow for these changes it was considered desirable to start with wheat the flour from which gave a dough having only moderate extensibility. A sample of Australian wheat was therefore chosen for the investigation.

The cleaned wheat had a test weight of 67 lb. per Imperial bushel and a moisture content of 11%. The flour milled from this wheat had the following analysis on a 14% moisture basis: ash, 0.40%; protein 8.5%; maltose figure, 1.65%.

Preparation of Samples. Part of the wheat was damped to moisture contents of 18.5% and 23.5% and these samples, together with some of the 11%-moisture wheat, were put into 3-liter glass bottles holding 1½- to 2-kg. lots. The bottles were kept at two temperature levels of 1°–2° C. and 20°–21° C. In addition, some of the original wheat was stored in a bag at room temperature.

The mouths of the bottles were closed with cotton-wool plugs. This method, which has been used by other workers (5), allows for air exchange between the inside and outside of the bottles, but also can result in some small change in the moisture content of the wheat.

The bottles were shaken daily to help maintain uniform moisture distribution, and at the same time the samples were inspected for any change in visual appearance due to mold growth.

Examination of Stored Wheat and Flour. The stored wheat was examined periodically, and various determinations on the wheat and flour were made as follows:

Milling characteristics. Before milling, the wheats were spread out on paper and air-dried for 72 hours, drying being facilitated by use of a fan. The wheat dried to about 14% moisture, was damped to 15.5%, and cold-conditioned for 18 hours. The wheat was milled on a Buhler laboratory mill and throughout the course of the investigation the conditions of milling, i.e., setting of the rolls, rate of feed, etc., were kept constant in order to reveal any differences in milling quality due to mold growth.

Moisture contents were determined by drying flour or coarsely ground wheat overnight at 100° C. Moist wheats were air-dried and the loss in weight was measured before grinding.

Other determinations. Fat, by extraction with boiling petroleum

ether (b.p. 40°–60° C.) under a reflux condenser; *Fat acidity*, by the method recommended by Zeleny and Coleman (25); *Mold counts*, by a method similar to the procedure given by Christensen (7); *Ash*, on a 5-g. sample incinerated overnight at 600° C. in a silica basin; *Thiamine*, by a thiochrome method based on the work of Ridyard (20). *Riboflavin* assays, by the microbiological method of Barton-Wright and Booth (2). *Nicotinic Acid*, by the microbiological method of Barton-Wright (1); *Color grade* figures were made with the Kent-Jones & Martin Flour Color Grader (17); *Diastatic activity* was measured by maltose figures determined as described in *Modern Cereal Chemistry* (16); *Nitrogen* was determined by the Kjeldahl-Gunning-Arnold method; converted to protein by the factor 5.7; *Test weights*, by the weight in g. of a 1-liter sample of wheat; the figure was converted to lbs. per Imperial bushel.

Water absorptions of flours were determined by the method of Halton (13). Three doughs made from 28 g. flour, 0.4 g. yeast, and different amounts of a 2.5% salt solution were fermented for 3 hours at 26.7° C. They were then extruded through a Simon Water Absorption Meter and from a plot of the log extrusion time against added salt solution the absorption corresponding to log 1.7, i.e., 50 seconds extrusion time, read off. This absorption was used for the extensometer and baking tests.

Physical dough tests were made by the method of Halton (13). Doughs made from 210 g. flour, 3 g. yeast, and the amount of salt solution found from the absorption test were fermented for 3 hours at 26.7° C.; then 75-g. test pieces were molded and, after a 30-minute rest period, stretched on a Simon Research Extensometer. The height of the curves gives a measure of the resistance to stretching, R, which is related to the elasticity of the dough; the length gives a measure of extensibility, E, or degree to which the dough stretches before rupture. The product figure of $R \times E/100$ is used as a measure of strength.

Baking Tests. For these, doughs were made from 140 g. flour, 2 g. yeast, and the amount of salt solution found from the absorption test. The doughs were fermented for 3 hours, molded, and tinned, and after 60 minutes' final proof, baked for 24 minutes at 220° C.

Fungal Cultures. These were obtained from the Commonwealth Mycological Institute and were carried on the following media:

Aspergillus niger	potato-dextrose agar
A. flavus	potato-dextrose agar
A. ochraceus	potato-dextrose agar
A. candidus	maize
A. amstelodami	malt extract containing 20% sucrose

Penicillium chrysogenum ....potato-dextrose agar
Alternaria tenuis .....potato-carrot agar

Inoculation of the experimental media was made by aseptically transferring some of the spores from slope cultures of the molds.

Experimental Cultures. The various fungi were inoculated into two media: (a) Czapek-Dox liquid medium, and (b) bran mash, prepared by autoclaving for 30 minutes at 20 lbs. pressure 20-g. aliquots of commercial bran with 70 ml. distilled water contained in 250-ml. Erlenmeyer flasks. These cultures were incubated 21 days at 26° C. and then harvested:

(a) Czapek-Dox cultures. The mycelia were separated by filtration and thoroughly washed with distilled water before being dried in a vacuum desiccator and ground through a 40-mesh sieve in a Wiley mill. The mycelia were added to the Australian flour at levels of 0.1% and 0.2% and the culture filtrates were added at a level of 15 ml. per 140 g.

(b) Bran cultures. The mixed bran and mycelia were dried down to approximately 14% moisture, either in an oven at 80° C. for 16 hours, or at room temperature. The various preparations were then ground through a 40-mesh sieve in a Wiley mill and added to the test flours at a level of 1%.

Extracts of the air-dried bran cultures were made by the method of Dirks and Miller (10), i.e., 5 g. extracted with 100 ml. 0.2% calcium chloride solution at 35° C. for 1 hour with stirring. The filtrates from these extractions were added to the Australian flour at a level of 20 ml. per 140 g. The residues from these extractions were dried at laboratory temperature, reground, and mixed with the test flour at a level of 1%.

The boiled extracts used in certain experiments were prepared as above and then steamed for 5 minutes at 100° C.

Control preparations were made in all cases using noninoculated bran which had been subjected to the same treatments as the experimental samples.

Flour. The cultures were added to an untreated flour milled from Australian wheat. Its analysis on a 14% moisture basis was: ash, 0.49%; protein, 8.4%; maltose figure, 1.65%.

Results

Effects of Mold Growth on Wheat. Since many of the changes noted in the stored wheat and flour milled from it are similar to those reported by Swanson (22) and other workers, they are discussed only briefly in this paper.

During the period of storage all the samples stored at 1°–2° C. showed little or no change. Of those stored at 20°–21° C., the 11% moisture sample remained substantially unchanged except for some improvement in the baking quality of the flour at the end of the period. Marked changes occurred, however, in the wheats having 18.5% and 23.5% moisture, and some of the data obtained on these samples and the flours milled from them are given in Table I.

TABLE I

CHANGES PRODUCED BY STORING HIGH-MOISTURE WHEAT AT 20°–21°C.

	Moisture Content of Wheat		
	11.0%	18.5%	23.5%
	Time of Storage (weeks)		
	0	8	4
Mold count (colonies per g.)	1,000	6,780,000	4,900,000
Aspergillus spp., %	64	79[a]	8
Penicillium spp., %	24	14[a]	88
Other molds, %	12	7[a]	4
Wheat fat, %[b]	2.06	1.63	1.68
Wheat fat acidity[c]	18.0	85.8	137.1
Flour yield, %	64.0	70.1	70.2
Break flour as % of total flour	14.4	17.0	18.8
Flour ash, %	0.49	0.70	0.74
Color grade figure	2.3	13.7	12.8
Flour fat, %	1.09	0.93	0.98
Flour fat acidity	16.2	49.2	63.7
Water absorption, %	56.1	34.6	31.0
Dough elasticity	410	140	180
Dough extensibility	12.5	2.5	3.5
Loaf volume, cc.	530	405	400

[a] After 6 weeks' storage.
[b] Fat, fat acidities, and ash figures given on dry-matter basis.
[c] Mg. KOH per 100 g. wheat or flour.

These data show that while the mold population on the wheat before storage was 1000 colonies per g., it increased to 6,700,000 on the 18.5% moisture wheat after 8 weeks' storage and to 4,900,000 on the 23.5% moisture sample after 4 weeks' storage. This increase in mold growth was accompanied by a fall in fat content and an increase in fat acidity, both for the wheats and the flours milled from them.

The data in Table I also show that the increase in mold growth resulted in a fall in test weight of the wheat but an increase in flour yield on milling. The increased flour extraction was due, at least in part, to greater bran fracture and resulted in a greater production of break

flour. The total flour obtained had a greater ash content and a higher color grade figure (i.e., poorer color), both reflecting increased bran content.

The data in Table II show that during storage of the wheat with 23.5% moisture at 20°–21°C. the thiamine and nicotinic acid in the wheat decreased whereas the content of riboflavin increased. The amount of these vitamins in the flour increased in storage, but in the case of thiamine and nicotinic acid this was probably due to the increased yield of flour bringing in parts of the grain rich in these vitamins (14).

TABLE II

CHANGES IN VITAMIN B CONTENT OF WHEAT AND FLOUR PRODUCED BY STORING WHEAT OF 23.5% MOISTURE AT 20°–21°C.

TIME OF STORAGE	THIAMINE		RIBOFLAVIN		NICOTINIC ACID	
	Wheat	Flour	Wheat	Flour	Wheat	Flour
Weeks						
0	4.58[a]	1.01	0.78	0.39	58.5	8.5
2	4.32	1.47	1.89	0.62	...	9.7
3	3.91	1.63	...	...	57.2	11.5
4	...	1.66	2.31	0.97	56.0	12.6

[a] All figures expressed in μg/g at 14% moisture.

No detailed study of changes in diastatic or proteolytic activity were made, but the following figures show that with increased mold growth there was a marked rise in reducing sugars and in the formol titration figures.

Storage time at 20°–21° C., 23.5% moisture	*Maltose*	*Formol titration*
	%	ml. 0.1 *N* NaOH/10 g flour
Before storage	1.6	0.32
2 weeks	2.75	0.48
3 weeks	3.35	0.96

These figures are probably of significance in relation to the changes that occurred in the baking quality of the flours. During the early stages of storage of the wheats, when the extraction of the flour was still unchanged, there was an improvement in baking quality, the loaves being larger in volume with darker crust color and softer, better developed crumb—changes which are similar to those resulting from increased amylase activity. With longer times of storage some deterioration in baking quality became apparent, and at the stage when the mold population became very high there was a very marked

drop in water absorption and a marked decrease in loaf volume. The bread then had a flat top with very smooth sides, sharp edges, and a very coarse crumb. The marked deterioration was similar to that produced by high proteolytic activity.

Fisher, Halton, and Carter (11) found that flour which had deteriorated markedly on storage improved the baking quality of sound untreated flour when added at the rate of 2%. Brückner (6) found that addition of moldy to normal flour in the ratio of 1 to 3 sometimes improved baking quality, but usually it deteriorated.

In this investigation the flour milled from the wheat stored for 3 weeks at 20°–21°C. with 23.5% moisture was added at the rate of 0, 1, 2, 4, and 8% to an Australian wheat flour and to a National flour milled from a mixed grist containing a high percentage of strong Canadian wheat. The effects produced were similar in kind with both flours. With increased additions of moldy flour there was a progressive fall in water absorption and dough elasticity and an increase in dough extensibility. These effects, together with the progressive increase in the smoothness of the loaf sides and coarsening of crumb structure found in the baking tests, could have resulted from the increased level of proteolytic activity introduced by the additions of moldy flour. On the other hand, the progressive darkening of crust color (up to 8% addition) and an increase in loaf volume and crumb softness (up to 2% addition) could be explained by an increase in amylase activity.

Effects Produced by Specific Mold Cultures on Dough and Bread Quality. (a) *Czapek-Dox cultures.* The separated mycelia and culture filtrates were tested by the methods outlined above. They had little or no effect on dough properties and produced only small changes in the bread. Crumb quality was best in the loaves containing the mycelia of *Aspergillus niger* and *Penicillium chrysogenum* and poorest with *A. ochraceus.* Addition of *P. chrysogenum* increased loaf volume slightly but made the crumb more open.

(b) *Bran cultures.* The effects produced by addition of air-dried bran cultures to flour are shown by the data in Table III, A. Addition of *Aspergillus flavus* and *A. ochraceus* caused a marked drop in water absorption and marked deterioration in both dough and bread properties. Addition of *A. amstelodami* and *A. candidus* resulted in a smaller reduction in water absorption, little change in dough properties, and some increase in loaf volume, accompanied, however, with a more open crumb. Addition of *P. chrysogenum* resulted in a drop in water absorption, little change in dough properties, and none in bread quality.

TABLE III

EFFECT OF BRAN CULTURES (AND EXTRACTS THEREOF) OF CERTAIN MOLDS, WHEN ADDED TO FLOUR, ON WATER ABSORPTION, DOUGH CHARACTERISTICS, AND LOAF CRUMB QUALITY

Mold	Absorption	Dough Elasticity (R)	Dough Extensibility (E)	Product Figure $\frac{R \times E}{100}$	Loaf Volume	Crumb Character Relative to Control
	%				cc.	
	A. Air-dried bran cultures					
None	57.2	370	12.0	44	490	.....
Aspergillus flavus	47.5	90	6.5	6	435	Dense; rubbery
A. ochraceus	47.5	80	7.0	6	430	Dense; rubbery
A. amstelodami	52.5	360	11.0	40	510	Open
A. candidus	53.7	310	12.5	39	525	Very open
Penicillium chrysogenum	55.7	330	12.5	41	495	Similar to control
A. niger	46.4	300	11.0	33	550	Finer; softer
Alternaria tenuis	55.0	450	10.0	45	490	Softer
	B. Oven-dried bran cultures					
None	57.9	330	13.5	45	480	.....
Aspergillus flavus	57.2	270	12.5	34	510	Very coarse
A. ochraceus	55.0	270	13.0	35	475	Closer
A. amstelodami	55.4	320	11.5	37	485	Like control
A. candidus	57.2	340	13.5	46	490	More open
Penicillium chrysogenum	56.8	320	13.0	42	500	More open
A. niger	55.4	310	13.5	42	545	Finer; softer
Alternaria tenuis	57.2	370	12.0	44	530	More open
	C. Calcium chloride extracts of air-dried bran cultures					
None	56.5	290	12.0	35	500	.....
Aspergillus flavus	42.5	110	3.5	4	425	Very coarse
A. ochraceus	42.5	70	4.5	3	500	Very coarse; hard
A. amstelodami	51.1	320	11.0	35	525	Slightly more open
A. candidus	49.6	290	11.5	33	550	More open
Penicillium chrysogenum	54.7	280	13.0	36	520	Slightly more open
A. niger	45.4	290	11.0	32	580	More open
Alternaria tenuis	54.5	310	13.5	42	555	More open
	D. Boiled calcium chloride extracts of air-dried bran cultures					
None	56.9	360	12.5	45	495	.....
Aspergillus flavus	56.5	350	11.5	40	495	More open
A. niger	56.5	390	12.5	49	530	More open
Alternaria tenuis	56.5	370	12.5	46	515	More open
	E. Residues of the calcium chloride extraction of air-dried bran cultures					
None	56.5	280	14.0	39	500	.....
Aspergillus flavus	54.4	290	11.0	32	450	Coarser; firmer
A. ochraceus	55.8	260	13.0	34	475	Coarser; firmer
A. amstelodami	55.8	280	11.0	31	490	Similar to control
A. candidus	55.8	340	12.0	41	495	Similar to control
Penicillium chrysogenum	56.5	340	12.0	41	490	Similar to control
A. niger	56.5	290	14.0	41	545	Finer; softer
Alternaria tenuis	56.5	350	11.0	39	505	Finer; softer

Addition of *Aspergillus niger* lowered water absorption even more than *A. flavus* or *A. ochraceus*. Its effects on dough and bread quality were, however, quite different. The dough softened markedly during fermentation and also during relaxation after molding, but its stretching characteristics were much superior to those of the doughs containing *A. flavus* and *A. ochraceus*. Bread quality was better than the control, loaf volume being larger and the crumb finer and softer. *Alternaria tenuis* produced a small reduction in water absorption, and the dough had improved elasticity (resistance to stretch) with reduced extensibility, an effect similar to that resulting from dough oxidation. Loaf volume was not increased, however, although the crumb was rather more developed than that of the control.

The bran cultures were also tested after being oven-dried, and the data in Table III, B, show that the effects produced were then much smaller than when the cultures were air-dried. This applies particularly to the ill effects of *Aspergillus flavus* and *A. ochraceus* on water absorption, dough properties, and bread quality and to the effect of *A. niger* on water absorption.

(c) *Extracts of bran cultures.* The calcium chloride extracts of the air-dried bran cultures had the same general effects although different in some details, as the cultures themselves. The data in Table III, C, show that the extracts of *Aspergillus flavus* and *A. ochraceus* produced a marked lowering of water absorption and marked deleterious effects on dough properties. *A. flavus* reduced loaf volume, but *A. ochraceus* did not. The crumbs of the loaves, although adversely affected, were different in character from those resulting from the addition of the unextracted cultures.

The extract of *Aspergillus niger*, like the culture itself, markedly lowered water absorption and had little effect on the stretching characteristics of the dough. In both cases loaf volume was increased, but, whereas the loaf containing the culture had a much finer crumb than the control bread, the loaf containing the extract had a more open crumb.

Some of the extracts were tested after being boiled and they then had much less effect on dough and bread properties, as can be seen from the data in Table III, D.

(d) *Residues from extracted bran cultures.* The bran culture residues after extraction with calcium chloride were also tested and the results obtained are given in Table III, E. In most cases the residues had much less effect than the cultures or extracts.

It is interesting to note that the residue of the culture of *Aspergillus niger* did not affect water absorption but increased loaf volume

and made the crumb finer and softer. The residue of the culture of *Alternaria tenuis*, like the culture itself, increased elasticity and reduced extensibility of the dough. In both cases loaf volume was not, however, increased, although the bread crumb was improved.

Discussion

The comparative inactivity of the separated moieties of the Czapek-Dox cultures of the various fungi is perhaps explicable by the varying metabolic activities and end products produced by such organisms when grown on such different media as the simple sugar-salts solution and the bran mash.

The effects produced by the bran cultures of the various molds varied markedly and cannot be fully explained. Comparison of the data for the air-dried and oven-dried cultures suggests that the substances responsible for the marked deleterious effects are heat-labile. The bran cultures were not heated in a manner which would ensure complete inactivation of enzymes, and so the residual effects obtained with the oven-dried cultures are explicable if the effects were due to proteolytic activity. The deleterious effects of *Aspergillus flavus* and *A. ochraceus* can probably be attributed to proteolytic enzymes of some type, since the factor (7) is evoked by growth on complex media, is extracted by weak calcium chloride solution, inactivated by boiling for 5 minutes, and partially inactivated at 80°C. when heated in a relatively dry condition.

Concomitantly with the evocation of proteolytic activity in the bran cultures, it is safe to assume the elaboration of amylases which are known to have an improving action on dough and bread quality (9, 15). Hence the degree of deterioration or improvement may well be a balance between the effects of proteolytic and amylolytic enzymes. In addition it must be remembered that while a high level of proteolytic activity causes deterioration, small additions produce different effects and can improve the baking quality of some types of flour.

While the marked deleterious effects of *Aspergillus flavus* and *A. ochraceus*, the milder deleterious effects of *A. amstelodami* and *A. candidus*, and the negligible effect of *Penicillium chrysogenum* could be due to proteolytic and amylolytic enzymes present in varying amounts, the behavior of *Alternaria tenuis* and *Aspergillus niger* is less easy to explain.

Alternaria tenuis is known to contribute most of the subepidermal mycelium found in sound, high-grade wheat (8) and is not known to cause any deterioration in stored grain. Throughout the tests reported

here it showed the tendency to increase the elasticity and reduce the extensibility of the dough. Such an effect could be produced by increased amylase activity, but in this case some increase in loaf volume would be expected. It could also be produced by substances acting in the same way as oxidizing agents. The absence of a corresponding increase in loaf volume could in this case be due to a low level of amylase activity. The action of this mold is interesting and may have some connection with the changes that occur during the maturation of grain or flour on storage. It merits further study.

The behavior of *Aspergillus niger* is also interesting. Its presence in the dough caused marked softening during fermentation, resulting in a considerable drop in water absorption. The dough also relaxed quickly after molding and would therefore be expected to have inferior baking quality. On the contrary, however, the loaves were larger than the controls and, in the case of the unextracted and extracted bran cultures, had finer crumb structure. The loaves containing the bran extracts, while of improved volume, had more open crumb than their controls.

In these experiments changes in crust color, which would have given some guide to changes in amylase activity, could not be followed owing to the colors imparted to the loaves by the bran cultures.

Literature Cited

1. Barton-Wright, E. C. The theory and practice of the microbiological assay of the vitamin-B complex, together with the assay of selected amino acids and potassium. Analyst **70:** 283–295 (1945).
2. Barton-Wright, E. C., and Booth, R. G. The assay of riboflavin in cereals and other products. Biochem. J. **37:** 25–30 (1943).
3. Bayfield, E. G., and O'Donnell, W. W. Observations on thiamin content of stored wheat. Food Research **10:** 485–488 (1945).
4. Bottomley, R. A., Christensen, C. M., and Geddes, W. F. Grain storage studies. IX. The influence of various temperatures, humidities, and oxygen concentrations on mold growth and biochemical changes in stored yellow corn. Cereal Chem. **27:** 271–296 (1950).
5. Bottomley, R. A., Christensen, C. M., and Geddes, W. F. Grain storage studies. X. The influence of aeration, time, and moisture content on fat acidity, nonreducing sugars, and mold flora of stored yellow corn. Cereal Chem. **29:** 53–64 (1952).
6. Brückner, G. Keimenergieprüfung und Kulturversuch zur Feststellung der Backeingung von Getreide. Z. ges. Getreidew. **20:** 262–268 (1933).
7. Christensen, C. M. The quantitative determination of molds in flour. Cereal Chem. **23:** 322–329 (1946).
8. Christensen, C. M. Fungi on and in wheat seed. Cereal Chem. **28:** 408–415 (1951).
9. Conn, J. F., Johnson, J. A., and Miller, B. S. An investigation of commercial fungal and bacterial alpha-amylase preparations in baking. Cereal Chem. **27:** 191–205 (1950).
10. Dirks, B. M., and Miller, B. S. Inactivation and removal of proteolytic enzymes from amylolytic supplements. Cereal Chem. **26:** 98–109 (1949).
11. Fisher, E. A., Halton, P., and Carter, R. H. Studies on the storage of wheaten flour. I. The influence of storage on the chemical composition and baking quality of flour. Cereal Chem. **14:** 135–161 (1936).

12. GILMAN, J. C., and SEMENIUK, G. Mold microflora in stored grain and its role in the determination process. Trans. Am. Assoc. Cereal Chemists **6:** 108–112 (1948).
13. HALTON, P. Significance of load-extension tests in assessing the baking quality of wheat flour doughs. Cereal Chem. **26:** 24–45 (1949).
14. HINTON, J. J. C., PEERS, F. G., and SHAW, B. The B vitamins in wheat. The unique aleurone layer. Nature **172:** 993–995 (1953).
15. JOHNSON, J. A., and MILLER, B. S. High levels of alpha-amylase in baking. I. Evaluation of the effect of alpha-amylase from various sources. Cereal Chem. **25:** 168–178 (1948).
16. KENT-JONES, D. W., and AMOS, A. J. Modern cereal chemistry (4th ed.), p. 451. Northern Publ. Co. Ltd.: Liverpool (1947).
17. KENT-JONES, D. W., and MARTIN, W. A photo-electric method of determining the color of flours as affected by grade, by measurements of reflecting power. Analyst **75:** 127–133 (1950).
18. LEAVITT, S., and LECLERC, J. A. Change in the composition of unground cereals during storage. Ind. Eng. Chem. **1:** 299–302 (1909).
19. MILNER, M., CHRISTENSEN, C. M., and GEDDES, W. F. Grain storage studies. V. Chemical and microbiological studies on "sick" wheat. Cereal Chem. **24:** 23–38 (1947).
20. RIDYARD, H. N. The determination of aneurin (Vitamin B_1) in uncooked wheat products. Analyst **74:** 18–24 (1949).
21. SORGER-DOMENIGG, H., CUENDET, L. S., CHRISTENSEN, C. M., and GEDDES, W. F. Grain storage studies. XVII. Effect of mold growth during temporary exposure of wheat to high moisture contents upon the development of germ damage and other indices of deterioration during subsequent storage. Cereal Chem. **32:** 270–285 (1955).
22. SWANSON, C. O. Some factors involved in damage to wheat quality. Cereal Chem. **11:** 173–199 (1934).
23. SWANSON, C. O. Effect of harvest conditions on a few quality factors in wheat. Cereal Chem. **13:** 79–90 (1936).
24. THOMAS, R. C. The role of certain fungi in the "sick-wheat" problem. Ohio Agr. Expt. Sta. Bimo. Bull. **22:** 43–45 (1937).
25. ZELENY, L., and COLEMAN, D. A. Acidity in cereals and cereal products, its determination and significance. Cereal Chem. **15:** 580–595 (1938).

EFFECTS ON BAKING POWDER BISCUITS OF FOUR FLOUR COMPONENTS USED IN TWO PROPORTIONS[1]

MARY V. ZAEHRINGER,[2] ALICE M. BRIANT,[3] AND CATHERINE J. PERSONIUS[4]

ABSTRACT

A commercial soft-wheat pastry flour and a commercial hard-wheat bread flour were separated into four fractions—gluten, starch, "amylodextrin," and water-solubles. A dark material representing about 5% of the flour was discarded during the fractionation and was not subsequently incorporated into the reconstituted flours.

More properties of reconstituted flours, and of doughs and biscuits made from the flours, were influenced by proportion than by source of the four major components: gluten, water-solubles, starch, and amylodextrin.

When proportions typical of a hard-wheat flour were used, flours had higher water-absorbing power and doughs were more compressible and more elastic than when proportions typical of a soft-wheat flour were used. Biscuits were darker in crust color, larger in volume, and less tender when made from flours mixed in hard-wheat proportions than when made from flours mixed in soft-wheat proportions.

When source of components varied, flours containing hard-wheat gluten had the greater sedimentation volumes. Biscuits containing hard-wheat gluten were larger and had paler and less tender crusts than biscuits containing soft-wheat gluten. Biscuits were smaller in volume and had less tender crusts and crumb when made from flour containing hard-wheat water-solubles. Biscuits had larger volumes and browner and more tender crusts when made with flour containing hard-wheat starch. Flours containing hard-wheat amylodextrin had greater sedimentation volumes, the doughs were less compressible, and the biscuits were smaller in volume but had browner crusts.

Several investigators, including Bechtel and Meisner (1), Finney (4), Harris and Sibbitt (6, 7), Prentice, Cuendet, and Geddes (12), Sandstedt, Jolitz, and Blish (13) and Yamazaki (14, 15), have used fractionation and reconstitution as a means of studying the functions of various components of wheat flours. No studies have come to our attention, however, in which the components of a soft-wheat and a hard-wheat flour were systematically interchanged in proportions typical of each type of flour, or in which baking-powder biscuits were made with reconstituted flours. We are reporting a study in which a commercial hard-wheat bread flour and a commercial soft-wheat pastry flour were each separated into four fractions—gluten, water-solubles, starch, and

[1] Manuscript received August 26, 1954. Contribution from the Department of Food and Nutrition, New York State College of Home Economics, Cornell University, Ithaca, New York. Taken from a thesis submitted to the Graduate Faculty of Cornell University by Mary V. Zaehringer, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1953.

[2] Present address: Department of Home Economics Research, Agricultural Experiment Station, University of Idaho, Moscow, Idaho.

[3] Associate Professor of Food and Nutrition.

[4] Professor of Food and Nutrition.

"amylodextrin." Thirty-two combinations of these eight fractions were made into reconstituted flours. Certain properties of the flours, and of doughs and biscuits prepared from the flours, were studied by means of objective measures and subjective judgments.

Materials and Methods

The flours were a commercial bleached hard-wheat bread flour (11.5% protein and 0.42% ash) and a commercial bleached soft-wheat pastry flour (7.7% protein and 0.39% ash), both reported on a 14% moisture basis.

Fractionation of the flours was carried out as follows: 200 g. of flour were mixed in a 3½-qt. bowl with sufficient tapwater to form a stiff dough, 116 ml. for the commercial pastry flour and 125 ml. for the commercial bread flour. The dough was kneaded 150 strokes, then submerged in 390 ml. of water in a 1-qt. bowl for 1 hour. The dough was then kneaded under the water until most of the starch had been washed out and a coherent mass of gluten was formed. The starch suspension was passed through a 40-mesh sieve and any particles of gluten remaining on the sieve were added to the gluten mass. The gluten was washed with six 33-ml. aliquots of water. The washed gluten was cut into thin slices, placed on sheets of cellophane, and frozen at −20°C. The wash-waters were passed through the sieve and added to the starch suspension.

The starch suspension was centrifuged at 2200 r.p.m. for 5 minutes. The supernatant liquid, containing the water-solubles, was poured into enamel pans to a depth of 1 cm. and frozen immediately at −20°C.

Three layers of material remained in the centrifuge tubes. The layers were carefully separated. The top layer was highly hydrated "amylodextrin" and the bottom layer, starch. Between these two was a thin layer of darker material. This material was somewhat cohesive in character and appeared to contain a small amount of granular material. Attempts to separate gluten, starch, or amylodextrin from this material were unsuccessful and it was discarded. It represented 5% of the flour. The starch and the amylodextrin were separately resuspended in water and recentrifuged, and a second separation of the fractions was made. The wash-water was discarded. The starch and the amylodextrin were spread on sheets of cellophane and frozen at −20°C.

The frozen fractions were lyophilized for 24 hours in a Stokes Dessivac Model 133-D. The dried gluten and water-solubles were ground in a Micro-Samplmill[5] using Screen No. 013 HBSS. The dried

[5] Pulverizing Machinery Co., Summit, New Jersey.

starch and amylodextrin were passed through a 40-mesh sieve. All lots of each fraction were combined and mixed for 5 minutes in the 40-qt. bowl of a Hobart Kitchen-Aid mixer, Model S230. The fractions were stored in covered jars, the gluten and water-solubles at −20°C. and the starch and amylodextrin at room temperature.

Reconstituted flours were prepared by recombining the fractions. There were 16 flours with the gluten, water-solubles, starch, and amylodextrin in the proportions obtained from the original commercial pastry flour, and 16 flours with the gluten, water-solubles, starch, and amylodextrin in the proportions obtained from the original commercial bread flour. Each group of flours included all possible combinations of source of the fractions except the dark material that was discarded. The proportions (Table I) were based on the yields of the fractions obtained from the two flours. It was assumed that the small differences in moisture content of the components were of no significance.

TABLE I

PROPORTION OF COMPONENTS IN RECONSTITUTED FLOURS[a]

	GLUTEN	STARCH	AMYLODEXTRIN	WATER-SOLUBLES
Hard-wheat proportions[b]	15.2	52.0	13.3	5.5
Soft-wheat proportions[c]	9.3	51.0	21.1	4.6

[a] 14% moisture.
[b] Based on yields of fractions obtained from the commercial bread flour.
[c] Based on yields of fractions obtained from the commercial pastry flour.

The reconstituted flours were prepared, on the sixth day before use, by sifting the weighed fractions into a cone-shaped mound, quartering the cone, and then resifting the alternate quarters into a mound. This procedure was repeated three times. The amount of flour mixed at one time was sufficient for the flour and dough tests and for one batch of biscuits. The flours were put in uncovered jars and, together with an open beaker of water, placed in a covered metal container. They were left at room temperature for 5 days, to bring the reconstituted flours to a moisture content approaching that of the commercial flours.

The design of the experiment was a 6×6 triple-lattice incomplete-block. An intrablock error was used to test the significance of differences between means attributable to main factors and interactions (8). This arrangement required 36 treatments. These treatments included the 32 reconstituted flours and the two commercial flours (a bread and a pastry flour) from which the experimental fractions were obtained.

To balance the design, two other commercial flours were included, but these are not considered in the results of this experiment. Inclusion of the original commercial pastry and bread flours provided a means of estimating the effect of fractionation and reconstitution.

Preparation of the biscuits. Each batch of biscuits was mixed from the following formula:

Formula for biscuits (% based on weight of flour)

	%
Flour	100.0
Salt	2.0
Baking powder, sodium aluminum sulfate-phosphate[a]	3.8
Hydrogenated vegetable fat	35.0
Water:	
Reconstituted flours	
HWP (hard wheat proportions)	51.0
SWP (soft wheat proportions)	46.0
Commercial flours	
Pastry flour	42.0
Bread flour	48.0

[a] Calumet Double Acting Baking Powder, General Foods Corporation, New York 17, New York.

The amounts of flour used were 170 g. of the reconstituted flours and 200 g. of the commercial flours. Preliminary investigations showed that biscuit doughs made with the commercial pastry flour and approximately 60% total moisture in the dough were similar in consistency to doughs made with the commercial bread flour and approximately 66% total moisture in the dough. The HWP doughs and doughs from the commercial hard wheat flour were prepared with approximately 66% total moisture in the dough. The SWP doughs and doughs from the commercial pastry flour were prepared with 60% total moisture in the dough.

The dry ingredients were sifted into a 2-qt. bowl; the fat was cut in with a pastry blender, using 50 strokes. The bowl was given a one-sixth turn between strokes and the sides were scraped after the fifteenth, the thirtieth, and the fiftieth stroke. The water was added all at once and was mixed in lightly with a fork, using 40 strokes. The dough was then formed into a ball, covered with a damp towel, and benched for 30 minutes. Following this period the dough was kneaded eight strokes and rolled into an oblong, 11.5 cm. by 22.9 cm. by 1.3 cm. A metal guide was used to control the thickness of the dough.

The biscuits were baked at 237°C. for 13 minutes. On removal from the oven, the biscuits were placed on wire racks to cool. Limitations of oven space and of personnel resulted in a delay of from 45

minutes to 1 hour and 45 minutes before the objective tests on the biscuits were made.

The flours, doughs, and biscuits were evaluated as follows:

A sedimentation test was made on each flour according to a modification (10) of the Zeleny procedure (11).

Measurements on the doughs required no additional manipulation of the dough and were made immediately. Compressibility and recoil of each dough were measured by means of a Precision Penetrometer. The compressibility of dough was recorded as the change in height of a piece of dough, 3.4 cm. in diameter and 1.3 cm. in height, caused by a load of 402.5 g. acting on the surface for 15 seconds. Elasticity of the dough was recorded as the change in height 15 seconds after the load had been removed.

The color of each batch of biscuits was rated on a seven-point scale ranging from 1 for a creamy color to 7 for a dark brown color. The mean of the scores for the lightest and the darkest biscuit from each batch was used to indicate the color of the batch. Although no attempt was made to correlate the color scores with consumer preference, biscuits with a score of 5 probably would be considered to have a desirable color.

Specific volume was measured by a modification of the rapeseed method using the batch of seven biscuits as a unit (3).

A panel of trained persons judged the tenderness of crumb and of crust and the flavor of the biscuits. The schedules of the judges did not permit them to judge the biscuits immediately after baking. The time of judging varied from 2½ to 6 hours after baking but was constant for any given judge. Variance due to time of judging, confounded with variance due to judges, was eliminated from the error variance in the statistical analysis of the data.

Results and Discussion

During fractionation of the commercial flours losses occurred, 10.7% for the bread flour and 9.2% for the pastry flour (Table I).

The dark material discarded during the starch-amylodextrin separation accounted for a loss of approximately 5%. The remaining losses were mechanical and unavoidable.

The moisture, nitrogen, and ether extract content of the original flours and of the fractions are given in Table II.

It will be noted that the two gluten fractions were alike in nitrogen content but that the HW water-solubles and the HW amylodextrin contained less nitrogen than the corresponding SW fractions.

The proportions of the fractions and their chemical analyses differ

TABLE II

PROTEIN AND ETHER EXTRACT CONTENT[a] OF FLOURS AND FLOUR COMPONENTS

COMPONENTS OF FLOURS	PROTEIN (N × 5.7)	ETHER EXTRACT
	%	%
Reconstituted flours		
Hard-wheat bread gluten	61.5	1.20
Hard-wheat bread starch	0.0	0.03
Hard-wheat bread amylodextrin	0.1	0.07
Hard-wheat bread water-solubles	13.7	0.40
Soft-wheat pastry gluten	61.1	3.01
Soft-wheat pastry starch	0.2	0.25
Soft-wheat pastry amylodextrin	0.7	0.39
Soft-wheat pastry water-solubles	18.5	2.09
Commercial flours		
Bread flour	11.6	1.19
Pastry flour	7.4	1.28

[a] 14% moisture basis.

TABLE III

COMPARISON OF COMMERCIAL HARD-WHEAT BREAD FLOUR AND COMMERCIAL SOFT-WHEAT PASTRY FLOUR WITH CORRESPONDING RECONSTITUTED FLOURS. MEAN[a] VALUES FOR MEASUREMENTS ON FLOURS, DOUGHS, AND BISCUITS

FLOUR	FLOURS	DOUGHS	
	Sedimentation Volume	Compressibility[b]	Recoil
	ml.	*mm.*	*mm.*
Commercial bread flour	28.7	9.09	2.40
Reconstituted bread flour	29.7	7.74	1.63
Difference	+1.0	−1.35**	−0.77**
Commercial pastry flour	12.0	8.84	1.57
Reconstituted pastry flour	6.0	7.60	1.13
Difference	−6.0**	−1.24	−0.44

	BISCUITS				
	Specific Volume[b]	Crust Brownness	Crust Tenderness[b]	Crumb Tenderness[b]	Flavor
	cc/g	*score*	*score*[c]	*score*[c]	*score*[d]
Commercial bread flour	1.92	4.0	4.8	4.8	3.9
Reconstituted bread flour	1.74	4.6	2.9	4.1	3.3
Difference	−0.18**	+0.6	−1.9**	−0.7	−0.6
Commercial pastry flour	1.95	3.3	5.1	4.8	3.7
Reconstituted pastry flour	1.75	2.0	4.5	4.5	3.1
Difference	−0.20**	−1.3**	−0.6	−0.3	−0.6

[a] Means of three replications.
[b] Means adjusted for block effects.
[c] Range for scoring: 6, very tender, to 1, very hard or very tough.
[d] Score: 4, pleasing; 3, flat.
** Difference between means significant at the 1% level.

in some respects from those reported by other workers (5, 12). These factors are, of course, influenced to a large extent by the method of fractionation.

The mean values for the measurements made on the original commercial bread and pastry flours, the corresponding reconstituted flours, and the doughs and biscuits made from these flours were compared to provide an estimate of the effect of fractionation and reconstitution. The mean values and the results of the statistical analyses are presented in Table III.

Fractionation and reconstitution of the commercial bread flour had no significant[6] effect on the sedimentation volume of the flour and on crust brownness, crumb tenderness, and flavor of the biscuits. There were significant decreases in the compressibility and elasticity of the doughs and in specific volume and crust tenderness of the biscuits. Fractionation and reconstitution of the commercial pastry flour had no significant effect on compressibility and elasticity of the doughs and on crust and crumb tenderness and flavor of the biscuits. There was a significant decrease in sedimentation volume of the flour and in volume and crust brownness of the biscuits.

Whenever fractionation and reconstitution effected significant differences in the measurements on the flours, doughs, or biscuits, the values were lower for the reconstituted flours and their products. The measurements of the flours, doughs, and biscuits that were affected were not the same for the two types of flour, with the exception of the specific volume of the biscuits. Fractionation and reconstitution increased the differences between the commercial pastry and the commercial bread flours for three measurements, decreased the differences for one measurement, and had no significant effect on four measurements. Conclusions based on these data should be tentative because each mean represents only three batches.

The means and the results of the statistical analyses of the data on the reconstituted flours and on doughs and biscuits made from them are given in Table IV.

The only quality not affected by the proportion and source of components was flavor, indicating that no one component from either source contributed a dominant flavor.

More of the characteristics of the reconstituted flours, and of the doughs and the biscuits prepared from the flours, were influenced by the *proportions* of the fractions than by the source of any individual fraction. This is not surprising, since gluten is considered the domi-

[6] Only differences significant at the 1% level were taken into account in the interpretation of the results.

TABLE IV

MEANS AND RESULTS OF STATISTICAL ANALYSIS OF DATA ON RECONSTITUTED FLOURS

SOURCE OF VARIANCE		FLOURS	DOUGHS		BISCUITS				
		Sedimentation Volume	Compressibility[a]	Recoil	Specific Volume[a]	Crust Brownness	Crust Tenderness[a]	Crumb Tenderness[a]	Flavor
		ml.	*mm.*	*mm.*	*cc/g*	*score*	*score*[b]	*score*[b]	*score*[c]
Proportion of components	H[d]	17.4**	8.70**	1.39**	1.86**	4.9**	3.3**	4.1**	3.3
	S	9.8	6.69	1.15	1.69	2.9	3.8	4.4	3.3
Source of components									
Gluten	H	20.0**	7.60	1.29	1.72**	3.6**	3.3**	4.2	3.2
	S	7.1	7.78	1.25	1.83	4.2	3.8	4.2	3.3
Water-solubles	H	13.9	7.62	1.27	1.74**	4.0	3.2**	4.1**	3.3
	S	13.2	7.76	1.27	1.81	3.8	4.0	4.4	3.2
Starch	H	13.3	7.57	1.27	1.79	4.0	3.8	4.3	3.3
	S	13.9	7.82	1.27	1.77	3.8	3.4	4.2	3.2
Amylodextrin	H	14.5**	7.28**	1.27	1.76**	4.2**	3.6	4.2	3.3
	S	12.7	8.10	1.27	1.80	3.6	3.6	4.3	3.3
All components[e]	H	21.8**	6.93**	1.46	1.65**	3.9	2.9**	4.2	3.4
	S	6.0	8.33	1.35	1.86	3.4	3.8	4.4	3.2

[a] Means adjusted for block effects.
[b] Range for scoring: 6, very tender, to 1, very hard or very tough.
[c] Score: 4, pleasing; 3, flat.
[d] H = hard wheat; S = soft wheat.
[e] These are means for three replications of two treatments.
** Difference between H and S means significant at the 1% level.

nant component of flour and there was a larger amount of gluten, a smaller amount of amylodextrin, and similar amounts of starch and water-solubles in the HWP flours.

The sedimentation volume of HWP flours was greater than that of SWP flours. This finding agrees with observations of others (16) concerning the effect of quantity of protein on sedimentation volume of flours. The hydrophylic nature of the amylodextrin (14) may have been an additional factor.

HWP doughs were more compressible and more elastic than SWP doughs. The larger amount of gluten and the smaller amount of amylodextrin in the HWP flours were probably the cause of the increased compressibility and elasticity of the doughs.

The specific volume of HWP biscuits was greater than that of SWP biscuits. The tenderness scores[7] for the crust and the crumb of HWP biscuits were less than those of SWP biscuits. The lesser tenderness of the HWP biscuits was expected, since the biscuits contained a larger quantity of the structure-contributing component, gluten. Also amylodextrin, present in smaller amounts in the HWP flours, has been reported to have a tenderizing influence on the crumb of bread (12, 13).

HWP biscuits had a darker crust color than SWP biscuits. Bertram (2) reported that increasing the amount of gluten in a flour provided more nitrogenous material for the "browning reaction" and resulted in a darker crust color in the baked product.

Considering the *source* of the individual components, the flours containing HW[8] *gluten* had considerably greater sedimentation volumes than the flours containing SW gluten. This is due to the greater water-absorbing power of HW gluten (5, 9).

Larger, browner, and more tender biscuits were obtained with the SW gluten than with the HW gluten. Although statistically significant, differences in the means were relatively small. The reasons for differences in crust color due to source of gluten were not determined in this study.

The *source* of the *water-solubles* had no effect upon sedimentation volumes of the flours. Evidently the differences in composition of the HW and SW water-solubles did not influence the water-imbibing or swelling properties of the flours. The compressibility and elasticity of the doughs were also unaffected by either the SW or the HW water-solubles.

Biscuits of smaller volume and of less tender crust and crumb were

[7] Range for scoring: 6, very tender, to 1, very hard or very tough.
[8] The source of components is designated as HW from hard wheat flour and SW from soft wheat flour.

obtained with HW than with SW water-solubles. These results indicate that the HW water-solubles had the lesser softening effect on the crumb and crust, though none of the dough measurements indicated a difference in the doughs. The color of the crust of the biscuits was not affected by the source of the water-solubles.

One significant interaction was observed between source of gluten and source of water-solubles. The SW water-solubles had more effect upon volume of biscuits when used with HW gluten than when used with SW gluten.

The *source* of *starch* had no effect on properties of the flours, doughs, and biscuits.

The *source* of *amylodextrin* affected some of the flours, doughs, and biscuits. Flours containing HW amylodextrin had a higher sedimentation volume than flours containing SW amylodextrin. Doughs containing HW amylodextrin were less compressible than doughs containing SW amylodextrin. It is probable that these differences are attributable to the greater water-absorbing power of the HW amylodextrin.

Biscuits containing HW amylodextrin were slightly smaller than biscuits containing SW amylodextrin, possibly because of a greater resistance of the HW doughs to expansion. The crusts of biscuits containing HW amylodextrin were darker than those containing SW amylodextrin. The tenderness of crust and crumb of biscuits was not affected either by the SW or HW amylodextrin.

The effects of using *all components* from a single *source* were examined. Flours containing all HW components had a considerably higher sedimentation volume than flours containing all SW components. Doughs containing all HW components were less compressible than doughs containing all SW components. These differences were probably a result of the combined effects of the greater water-absorbing power of the HW gluten and the HW amylodextrin. The greater volume and crust tenderness of biscuits made with all SW components reflect a summation of the effects of the separate components, several of which made some contribution to these differences. The source of all components had no effect on crust color and crumb tenderness.

Significant interactions between proportion and source of a component were observed for only three of the measurements. Where observed, they generally reflected the trends with the separate variables and point up some irregularities in extent of the response.

Interactions between proportion and source of all components were observed only for sedimentation volume of the flours and crust tenderness of the biscuits.

Acknowledgments

The authors gratefully acknowledge the cooperation of Dr. Paul E. Ramstad in making available the Dessivac for drying the flour fractions; of Dr. Frances A. Johnston for supervising the nitrogen determinations; and of Dr. John K. Loosli for supervising the fat determinations.

The authors also wish to acknowledge the technical assistance of Mrs. Anna Rogers Willman in the preparation of the flour fractions, and of Miss Mary C. McAuley for the determination of sedimentation volume.

Literature Cited

1. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. I. Fractionation of flour and preparation of bread. Cereal Chem. **31:** 163–170 (1954).
2. Bertram, G. L. Studies on crust color. I. The importance of the browning reaction in determining the crust color of bread. Cereal Chem. **30:** 127–139 (1953).
3. Cathcart, W. H., and Cole, L. C. Wide range volume-measuring apparatus for bread. Cereal Chem. **15:** 69–79 (1938).
4. Finney, K. F. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. **20:** 381–396 (1943).
5. Gortner, R. A., and Doherty, E. H. Hydration capacity of gluten from "strong" and "weak" flours. J. Agr. Res. **13:** 389–418 (1918).
6. Harris, R. H., and Sibbitt, L. D. The comparative baking qualities of starches prepared from different wheat varieties. Cereal Chem. **18:** 585–604 (1941).
7. Harris, R. H., and Sibbitt, L. D. The comparative baking qualities of hard red spring wheat starches and glutens as prepared by the gluten-starch blend baking method. Cereal Chem. **19:** 763–772 (1942).
8. Hohemeyer, P. G., Clem, M. A., and Federer, W. T. Punched card and calculating machine methods for analyzing lattice experiments including lattice squares and the cubic lattice. Research Bulletin 347. Iowa State College Agricultural Experiment Station (April, 1947).
9. Markley, M. C. The colloidal behavior of flour doughs. III. Studies upon the properties of flour-starch-water systems. Cereal Chem. **15:** 438–444 (1938).
10. McAuley, Mary C., Zaehringer, Mary V., and Briant, Alice M. Modification of sedimentation test for soft wheat and reconstituted flours. Cereal Chem. **30:** 446–447 (1953).
11. Micka, J. Zeleny's sedimentation test for biscuit and cracker flours. Trans. Am Assoc. Cereal Chemists **10:** 188–192 (1952).
12. Prentice, N., Cuendet, L. S., and Geddes, W. F. Studies on bread staling. V. Effect of flour fractions and various starches on the firming of bread crumb. Cereal Chem. **31:** 188–206 (1954).
13. Sandstedt, R. M., Jolitz, C. E., and Blish, M. J. Starch in relation to some baking properties of flour. Cereal Chem. **16:** 780–792 (1939).
14. Yamazaki, W. T. The fractionation and reconstitution of soft winter wheat flours. Ph.D. thesis, Ohio State University (1950).
15. Yamazaki, W. T. The concentration of a factor in soft wheat flours affecting cookie quality. Cereal Chem. **32:** 26–37 (1955).
16. Zeleny, L. A simple sedimentation test for estimating the bread-baking and gluten qualities of wheat flour. Cereal Chem. **24:** 465–475 (1947).

OBSERVATIONS ON THE INFLUENCE OF TEXTURATION, OCCLUDED GAS CONTENT, AND EMULSIFIER CONTENT ON SHORTENING PERFORMANCE IN CAKE MAKING[1]

S. W. THOMPSON[2] AND J. E. GANNON[2]

ABSTRACT

Conventional nonemulsifier and emulsifier shortenings, texturated with and without the usual 10% occluded gas content and also solidified by slow cooling without agitation, were tested in several cake formulas and cake-mixing methods. The effect of monoglyceride level in the texturated emulsifier shortening was determined in the same tests.

Emulsified gas in the shortening contributed to performance in creaming-method pound cakes and in certain layer cakes, but its omission could be compensated for by additional mixing of the batter. Texturation of the shortening was an important factor in the quality of cakes produced by most formulas. Good cakes were obtained with untexturated shortening in certain formulas by considerable additional mixing of the batter. The optimum level for the conventional type of monoglyceride in shortening was about 3%, but depended on the cake formula and was not very critical in the 2–4% range.

Commercial shortenings normally contain 10–12%, by volume, of gas which is dispersed in very fine bubbles (2–10 μ in diameter) throughout the mass. The primary purpose in adding this gas is to contribute an attractive white appearance to the shortening, but there seems to be a fairly common popular belief that the gas also contributes significantly to cake-baking performance. This thought may have been fostered to some extent by the work of Carlin (2), which demonstrated the significance of the air bubbles in the fat *in the batter*. However, Dunn and White (3) showed some years earlier that omission of occluded gas in shortening could be compensated for by extra creaming in making pound cake by the creaming method.

There is relatively little information in the literature on the relation between texturation[3] or plasticizing of fats and their cake-making properties. Bailey (1), in discussing the creaming and cake-making action of shortenings, points out that if fat crystals are large, as in lard or in fats which are allowed to solidify slowly, cake action is poor. Commercial finishing of hydrogenated vegetable oil shortenings produces a very fine crystal structure, and this would appear to be in some degree responsible for their superior cake-making performance.

A number of investigators have studied the effect of the mono-

[1] Manuscript received June 15, 1955. Presented at the annual meeting, St. Louis, Missouri, May 1955.
[2] Lever Brothers Company, Edgewater, N. J.
[3] The processing step which converts shortening from liquid to solid phase and may also incorporate a finely divided gas phase.

glyceride (MG) level in shortening on its cake action. Carlin (2) found that increasing amounts up to 8 or 9% of emulsifier in the shortening was beneficial in white layer cakes, and noted a decrease in cake volume at still higher emulsifier levels. Presumably he used a commercial mono-diglyceride concentrate prepared from partially hydrogenated vegetable oil and the actual MG levels were somewhat less than half the indicated emulsifier levels.

Kuhrt and Welch (5) chose a 130%-sugar, yellow loaf cake for their investigation and found that with a commercial, partially hydrogenated, vegetable oil mono-diglyceride mix as the MG source, the maximum effect from the monoester was reached at 4% (pure MG, shortening basis), but no adverse effect occurred as the MG level in the shortening was increased up to 9%. With distilled monoglycerides of unhardened cottonseed oil they obtained optimum cake volumes at MG levels in the shortening, varying from 1% when the shortening level in the cake was 50% of the flour weight, up to 3% when shortening level was only 32.5% of the flour weight. With this distilled unsaturated acid MG there was a fairly sharp drop in cake volume as the optimum MG level was exceeded.

In a 130% white cake formula, Favor *et al.* (4) found up to 4% mono-diglyceride emulsifier (shortening basis) desirable, with no effect from further increases up to 14%. Presumably their mono-diglyceride mixture was 40–45% MG prepared from a partially hydrogenated vegetable oil.

Materials and Methods

The cake formulas used are given in Table I.

TABLE I

CAKE FORMULAS

Ingredient	Type of Shortening and Type of Cake						
	Nonemulsifier			Emulsifier			
	Yellow Pound	Yellow Layer	White Layer	Yellow Pound	Yellow Layer A	Yellow Layer B	White Layer
	Parts	*Parts*	*Parts*	*Parts*	*Parts*	*Parts*	*Parts*
Flour	100	100	100	100	100	100	100
Sugar	100	100	100	120	120	110	125
Shortening	50	45	40	70	45	40	50
Eggs	50	40	55	70	50	40	60
Milk	55	85	60	50	100	110	95
Baking powder	...	6	6	...	6	4.5	6
Salt	3	3	3	3	3	3	3
Cake scaling wts., oz.	40	11	12	40	12	8.5	13

Reconstituted nonfat milk solids, frozen eggs (whole or whites depending upon cake formula), fruit-fine sugar and, except in one cake, best-quality cake flour were used. The specifications for the top-grade cake flour were: protein content 7.5–7.9%, ash 0.32–0.35%. Less expensive soft-wheat flours with a lower protein and higher ash content are sometimes encountered in the baking trade, and their use makes some cake formulas more sensitive to the emulsifier content of the shortening. To illustrate this effect, a 7% protein flour, obtained by mixing the high-quality cake flour noted above with West Coast soft-wheat flour, was used in yellow layer formula B.

Batters were prepared in a 12-qt. Hobart machine Model A120 by both the two-stage blending and three-stage creaming methods for the nonemulsifier shortening cakes and by both two-stage and three-stage blending methods for the emulsifier shortening cakes. In the two-stage blending method the shortening, other dry ingredients, and a portion of the milk were mixed in the first stage, with the remaining liquids added in the second. In addition to the foregoing a modified two-stage method was used in making up the emulsifier-shortening pound cake formula. In this modified method, four-sevenths of the eggs were included in the first stage. Mixing times and speeds are listed with cake results. Cake volume measurements serve as a satisfactory index of the effect of the variables reported in the present work even though other qualities are also affected.

Cake volumes were determined in duplicate by the seed displacement method. The specific volume (reciprocal of specific gravity) was determined on all batters.

For investigating the effect of the emulsified gas in shortening, production samples of both emulsifier and nonemulsifier shortenings were melted and replasticized in a laboratory model Votator under identical chilling conditions with and without 10% emulsified gas addition. The stock for the variation without gas was fed from a tank under vacuum to avoid production of any emulsified gas from dissolved gas, on release of the shortening to atmospheric pressure from the Votator system.

Two untexturated shortening variations were produced by allowing melted stock to cool and solidify slowly in a room at 10°C. (50°F.) and in a room at 21.1°C. (70°F.). For the emulsifier level tests, a partially hydrogenated vegetable oil mono-diglyceride concentrate, 45% MG by analysis, more or less typical of the material used in many commercial shortenings, was texturated with an equal proportion of nonemulsifier shortening in the laboratory-size Votator. This material was scaled

with appropriate proportions of nonemulsifier shortening to obtain the range of MG levels desired for the cake tests.

Effects of Shortening Texturation and Occluded Gas Content

Table II shows the results obtained with conventionally texturated, unaerated-texturated, and untexturated nonemulsifier shortenings. Lack of emulsified gas in the shortening had no adverse effect in the blending-method pound cake, but in the creaming-method it depressed the creaming volume considerably and the batter and cake volumes slightly. A test was made in which the second-stage creaming time was extended 7 minutes at low speed. This brought the creaming volume to 1.55 and the subsequent batter volume to 1.32, and gave as large a cake as the aerated shortening.

TABLE II

RESULTS OF NONEMULSIFIER-SHORTENING CAKE TESTS

SHORTENING TEXTURATION	STANDARD	UNAERATED	CHILLED AT 10° C.	CHILLED AT 21.1° C.
	Pound cake – 2-stage blending method (3½ minutes M, 3½ minutes L[a])			
Batter, specific volume	1.35	1.39	1.08	1.04
Cake volume (cc.)	2965	3050	2440	2340
	Pound cake – 3-stage creaming method (3½ minutes L, 4 minutes L, 3½ minutes L)			
Cream, sp. vol.	1.55	1.35	1.53	1.50
Batter, sp. vol.	1.33	1.27	1.17	1.14
Cake volume (cc.)	2930	2860	2600	2535
	Yellow layer – 2-stage blending method (4 minutes L, 4 minutes L)			
Batter, sp. vol.	1.22	1.22	1.07	1.04
Cake volume (cc.)	1000	990	985	990
	Yellow layer – 3-stage creaming method (4 minutes L, 4 minutes L, 4 minutes L)			
Batter, sp. vol.	1.37	1.33	1.28	1.26
Cake volume (cc.)	1020	1030	1030	1020
	White layer – 2-stage blending method (4 minutes L, 4 minutes L)			
Batter, sp. vol.	1.17	1.19	1.02	0.96
Cake volume (cc.)	945	955	900	885

[a] M = medium speed, L = low speed.

Both of the untexturated shortenings gave low batter volumes and poor cakes in the blending-method pound cake. Extending the mixing time in either the first or second stages failed to improve batter volume appreciably.

It is rather interesting that in the creaming method the untexturated fats gave nearly normal creaming volumes, but the batter and cake volumes were low although somewhat better than those obtained by the blending method. Extending the second- and third-stage mixing times singly or in combination failed to produce a satisfactory cake with the untexturated shortening in this method.

Omission of the air from the shortening did not affect any of the nonemulsifier-shortening layer cakes significantly. While the batter volumes were much lower, the untexturated shortenings made as good yellow layers by both mixing methods as the texturated shortenings. The latter, however, gave better white layer cakes. A supplementary white layer test was made with the untexturated shortening (21.1°C.

TABLE III

RESULTS OF EMULSIFIER-SHORTENING CAKE TEST

SHORTENING TEXTURATION	STANDARD	UNAERATED	CHILLED AT 10° C.	CHILLED AT 21.1° C.
	Pound cake — 2-stage blending method (3½ minutes M, 3½ minutes L[a])			
Batter, specific volume	1.23	1.21	1.05	1.01
Cake volume (cc.)	2920	2875	2505	2355
	Pound cake — modified 2-stage method (4 minutes M, 4 minutes L)			
Batter, sp. vol.	1.34	1.32	1.12	1.05
Cake volume (cc.)	3145	3115	2555	2345
	Pound cake — 3-stage blending method (2 minutes M, 4 minutes M, 3 minutes L)			
Batter, sp. vol.	1.26	1.26	1.19	1.12
Cake volume (cc.)	2950	2950	2715	2600
	Yellow layer A — 2-stage blending method (4 minutes L, 4 minutes L)			
Batter, sp. vol.	1.03	1.00	.94	.92
Cake volume (cc.)	1040	1015	990	960
	Yellow layer A — 3-stage blending method (4 minutes L, 4 minutes L, 4 minutes L)			
Batter, sp. vol.	1.01	0.97	0.97	0.94
Cake volume (cc.)	1095	1070	1020	1020
	White layer — 2-stage blending method (4 minutes L, 4 minutes L)			
Batter, sp. vol.	1.05	1.06	0.97	0.94
Cake volume (cc.)	1030	1030	945	920
	White layer — 3-stage blending method			
Batter, sp. vol.	1.06	1.02	1.03	0.98
Cake volume (cc.)	1035	1015	1000	970

[a] M = medium speed; L = low speed.

chilled) in which the last-stage mixing was extended several minutes. When this failed to raise batter volume, an additional ½ oz. of baking powder was added and the cake baked from this batter had a volume of 920 cc.

Volumes of cakes made with emulsifier shortening variations are in Table III. Although the 2-stage pound cake methods gave slightly lower volumes with the unaerated shortenings, the differences are of doubtful significance. In these pound cake methods, cake volume is definitely a function of batter volume, one point in batter volume being equivalent to about 20 cc. of cake volume in the 2800–3000 cc. volume range. Batter volume increases one to two points per minute of low-speed mixing, so that cake volume can be adjusted to any desired level and lack of aeration in the shortening can be easily offset.

The untexturated shortenings failed to make good pound cakes although they performed better in the three-stage method than in the others. Batters containing the untexturated shortening did not pick up air readily with extra mixing. A two-stage batter containing the fat which had been solidified at 21.1°C. was given 20 minutes' additional time at medium speed and showed only a 4-point increase in specific volume.

In the emulsifier-type yellow layer cakes, the unaerated shortening yielded marginally poorer and the untexturated shortenings appreciably poorer results. This is in contrast to the results in yellow layer cakes with the nonemulsifier shortening (Table II), where neither aeration nor texturation of the shortening was of any significance. Since the cake volumes appeared to correlate with the batter volumes, a test was made in which a two-stage batter containing the untexturated shortening, solidified at 21.1°C., was mixed an additional 20 minutes. This brought the batter volume to 1.02 and resulted in a 1020-cc. cake of good quality, thereby largely compensating for the lack of texturation.

In the white layer cake tests with the emulsifier shortenings, the lack of emulsified gas in the shortening did not show up when the cake was mixed by the two-stage method and was barely apparent in the three-stage method. The cakes made with the fat solidified at 10.0°C. were down somewhat in volume and those made with the fat which was allowed to set up at 21.1°C. were still smaller.

Again it was found that the lack of texturation could be offset, but only by considerable additional mixing of the batter. Twenty minutes extra at medium speed in the last stage of the first method (using shortening solidified at 21.1°C.) were required to bring the batter volume to 1.03, and this resulted in a cake equal to that obtained with

the regularly texturated shortening.

Extra mixing in the last stage only of the three-stage method was ineffective, but 4 minutes extra at low speed in the second stage, plus 10 minutes extra at medium speed in the third stage, compensated for lack of texturation in the shortening.

In general, the cake tests in which there was a difference between texturated and untexturated shortening also showed a difference between shortening solidified at 10.0°C. and shortening solidified at 21.1°C., in favor of the former. Presumably the treatment at 10.0°C. produced smaller crystals and showed a slight tendency to approach the effect of Votator chilling.

Effects of Monoglyceride Level

The results in Table IV demonstrate the effect of the shortening monoglyceride content in the two-stage, 120%-sugar pound cake. As the MG was increased above 2%, the batter and cake volumes began to decrease but only a few minutes extra mixing was required to counteract this effect of the higher MG levels. In pound cake the use of emulsifier shortening benefits eating quality and keeping quality rather than being reflected in cake volume.

In the yellow layer cake formula A utilizing high-grade cake flour (Table V), batter volume and cake volume showed a strong inverse relationship: as the MG in the shortening was increased the batter volume dropped but the cake volume rose. Minimum batter volume and maximum cake volume were reached at the 5% MG level in the shortening, although the change in cake volume over the 3% to 5% MG-level range was hardly significant. As the MG was increased to 10

TABLE IV

EFFECT OF MONOGLYCERIDE LEVEL IN POUND CAKE
(Two-stage blending method)[a]

Monoglyceride in Fat	Standard Mixing Time		Extra Mixing Time	
	Batter Volume	Cake Volume	Batter Volume	Cake Volume
%				
0	1.34	2900	.....	.....
1	1.34	2965	.....	.....
2	1.33	2965	.....	.....
3	1.27	2910	1.31[b]	3000
4	1.25	2845	1.36[c]	3015
5	1.22	2805	1.33[d]	3030

[a] 3½ minutes medium speed, 3½ minutes low speed.
[b] Extra mixing time 3 minutes, second stage.
[c] Extra mixing time 4 minutes, second stage.
[d] Extra mixing time 5 minutes, second stage.

TABLE V

EFFECT OF MONOGLYCERIDE LEVEL IN YELLOW LAYER CAKE A
(Two-stage blending method)[a]

Monoglyceride in Fat	Standard Mixing Time		Extra Mixing Time[b]	
	Batter Volume	Cake Volume	Batter Volume	Cake Volume
%				
0	1.23	1020	....	....
1	1.16	1040	....	....
2	1.07	1055	....	....
3	1.05	1070	....	....
4	1.01	1070	1.03	1100
5	0.99	1080	1.02	1080
10	1.01	1005	1.01	1035
20	1.08	945	....	....

[a] 4 minutes medium speed, 4 minutes low speed.
[b] 15 minutes extra at low speed, second stage.

and 20%, batter volume increased slightly but cake volume dropped off quite sharply.

As compared to the yellow layer A formula, the yellow layer B formula (Table I) gives a somewhat cheaper and more sensitive cake. A flour of lower grade is used, eggs and shortening proportions are less, and although the sugar is only 110% of the flour, the total liquids (eggs plus milk) remain high. This cake was baked in 7-inch layers rather than 8-inch like the other layer formulas. The results obtained varying the MG level of the shortening used in formula B are in Table VI.

Batter volumes follow the same trend as in Table V, passing through a minimum at 5% MG in the shortening. However, in this case the cake volume failed to drop and, if anything, increased slightly as the MG level was raised from 5 to 10% in the shortening. The increase in cake volume due to the introduction of 3% MG in the

TABLE VI

EFFECT OF MONOGLYCERIDE LEVEL IN YELLOW LAYER CAKE B
(Three-stage blending method)[a]

Monoglyceride In Fat	Batter Volume	Cake Volume	Monoglyceride In Fat	Batter Volume	Cake Volume
%			%		
0	1.19	650	4	0.96	735
1	1.09	680	5	0.94	735
2	1.00	705	6	0.97	740
3	0.97	735	10	0.99	750

[a] 3 minutes low speed, 4 minutes low, 4 minutes low.

TABLE VII

EFFECT OF MONOGLYCERIDE LEVEL IN WHITE LAYER CAKE
(Two-stage blending method)[a]

Monoglyceride In Fat	Batter Volume	Cake Volume	Monoglyceride In Fat	Batter Volume	Cake Volume
%			%		
0	1.21	840	4	1.04	1065
1	1.20	970	5	1.00	1050
2	1.19	1040	10	1.01	1050
3	1.07	1070	20	1.03	1040

[a] 4 minutes low speed, 4 minutes low.

shortening was 13% for formula B as against only 6% for formula A.

Finally, the effect of MG level in a 125% white layer cake is shown in Table VII. Batter volumes followed the usual trend reaching a minimum at 5% MG. Cake volume reached a maximum at the 3% MG level but dropped off only slightly as the proportion in the shortening was raised to 20%.

Among the formulas used in these tests this white layer cake shows the greatest benefit from emulsifier. Only 1% MG addition to the shortening resulted in a 15% volume increase, whereas with 3% MG the volume increase was 27%.

Literature Cited

1. BAILEY, A. E. Industrial oil and fat products (2nd ed.). Interscience: New York (1951).
2. CARLIN, G. T. A microscopic study of the behavior of fats in cake batters. Cereal Chem. **21:** 189–199 (1944).
3. DUNN, J. A., and WHITE, J. R. The leavening action of air included in cake batter. Cereal Chem. **16:** 93–100 (1939).
4. FAVOR, H. H., CROSSLAND, L. B., and BEASLEY, H. K. Emulsifiers in prepared cake mixes. Cereal Chem. **30:** 435–441 (1953).
5. KUHRT, N. H., and WELCH, EILEEN A. Molecularly distilled monoglycerides. II. Cake baking experiments. J. Am. Oil Chem. Soc. **27:** 344–346 (1950).

ESTIMATION OF PROTEIN IN WHEAT AND FLOUR BY ION-BINDING[1]

DOYLE C. UDY[2]

ABSTRACT

The wheat proteins react with the disulfonic acid dye, orange G, at pH 2.2 to form an insoluble complex. The amount of dye bound per g. of sample may be used to provide an accurate estimate of protein content. In practice, the estimate is based on the concentration of unbound dye, as measured colorimetrically using a light filter (470 mμ).

For the experimental conditions employed, the equation relating protein content (N × 5.7), P, to the concentration of unbound dye, C, was P = 44.47 − 50.00 C for 128 samples of wheat representing more than 50 varieties containing from 6.2 to 16.0% protein. For 218 samples of straight-grade flour containing from 4.6 to 15.2% protein, the equation was P = 40.92 − 45.54 C. The correlation coefficients between bound dye and protein content were 0.992 and 0.997 for wheat and flour respectively; the standard errors of estimate of protein content from the concentrations of unbound dye were 0.22% for wheat and 0.20% for flour.

Starch and bran bind significant amounts of the dye.

Recent studies (7) yielded information concerning the dye-binding capacities of wheat proteins which indicated that the reaction between certain dyes and wheat proteins might be a useful analytical tool for quantitative estimation of the protein contents of whole wheat and wheat flour. This paper presents data utilizing such a reaction and describes a method for estimating protein in wheat products.

At a low pH the dissociated sulfonic acid groups of the dye, orange G, react with basic groups on protein molecules to form an insoluble protein-dye complex. This reaction was the basis of a method used by Fraenkel-Conrat and Cooper (2) to determine the number of basic groups on several different proteins or protein fractions.

The method proposed here is based on this reaction and an assumption that the several proteins of wheat are distributed in a regular manner among varieties. A regular distribution was indicated in a previous study (7), and the work reported here gives support to this supposition. Measurements of the direct binding of hydrogen ions by careful pH readings[3] gave results that were accurate to within 10% on several flour samples, but this approach was not pursued further inas-

[1] Manuscript received April 4, 1955. Contribution from the Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Washington Agricultural Experiment Stations, State College of Washington; and the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Scientific Paper No. 1330, Washington Agricultural Experiment Stations, Pullman.

[2] Associate Chemist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Agricultural Experiment Stations, Pullman, Washington.

[3] Unpublished results of this Laboratory.

much as the dye-binding technique appeared to be much more accurate.

Materials and Methods

Wheat samples were obtained from several experiment stations in the Pacific Northwest and represent 58 varieties plus 34 selections of unknown parentage. The range of protein content varied from about 5% to 16%. Each sample was uniformly milled on an experimental Buhler Mill to obtain a straight-grade flour. A Hobart Mill was used to prepare the whole-wheat samples. A total of 128 whole-wheat samples and 218 flour samples were tested by the procedure outlined below.

Briefly, the amount of dye bound per g. of sample and its protein content ($N \times 5.7$) were plotted. From the regression line obtained, it is possible to estimate the protein content of any sample by measuring the concentration of unbound dye. The amount bound is obtained by difference from the known excess of dye added. A micro-Kjeldahl method (5) was used to determine the protein. Duplicate analysis agreed within $\pm 0.1\%$ protein by the Kjeldahl procedure and bound the same amount of dye within ± 0.1 mg.

Measurement of Dye-Binding Capacity. Orange G, a water-soluble disulfonic acid dye manufactured by Allied Chemical and Dye Corporation, New York, N. Y., was used after drying at 80°C. Different lots of the dye had dye contents ranging from 93.5% to 97% (as determined from their chromogenic value) when compared with the pure dye obtained by recrystallization from 90% ethyl alcohol. It was convenient to use a buffered[4] solution of the recrystallized dye as a reference standard. This solution contained 100.0 mg. of pure dye per 100.0 ml. of solution at 20°C. All measurements were made under the same conditions of colorimeter operation. One ml. of a 10% alcoholic solution of thymol per 8 liters of buffered dye solution prevented mold growth.

Method for Flour Samples. Flour samples of 600 mg. were weighed, then placed in 50-ml. polyethylene centrifuge tubes. A volume of 25.00 ml. of orange G dye solution was added from an automatic pipette. Working solutions were prepared from the unrecrystallized stock dye to contain the equivalent of 100.0 mg. of pure dye per 100.0 ml. of solution and buffered to pH 2.2 with McIlvaine's buffering reagents.[4] Smaller flour samples may be used with smaller volumes of dye solution by keeping the ratio of volume to sample weight constant.

[4] A solution of pH 2.2 is obtained with 20.7 g. of citric acid monohydrate and 1.44 g. of disodium phosphate dodecahydrate per liter.

After addition of the dye, the tubes were stoppered with polyethylene caps and rocked for 15 minutes to allow complete reaction between the cationic groups of the protein molecules and the anionic dye molecules. The insoluble protein-dye complex and other insoluble components of flour were separated from the solution by centrifuging for 5 minutes at 4000 r.p.m. or until a clear supernatant solution was obtained.

An Evelyn photoelectric colorimeter in conjunction with a 470mμ filter and a special short-light-path absorption cell[5] were used for measuring the unbound dye concentration in the supernatant solution. The readings on the sensitive galvonometer were estimated to the nearest 0.1% transmission. A precision of $\pm 1\%$ in the amount of unbound dye was easily attained. This corresponds to about one-tenth of a protein percentage unit.

Since the protein content of the flour sample is related to the concentration of unbound dye, it can be read directly from a prepared table. Such a table was prepared from the equation relating protein content and unbound dye concentration (developed in the section on results) and included a column relating dye concentration with either absorbance or percent transmission. The latter relationship should be carefully determined for the particular absorption cell used and other specific conditions of colorimeter operation. A periodic check of this relationship must be made to ensure consistent colorimeter readings.

Method for Whole Wheat. Ground whole wheat was analyzed for protein in the same manner as flour except for the ratio of sample weight to volume of dye solution and the period of shaking. The same volume of dye of the same concentration was added to 500 mg. of ground wheat. A reaction (or shaking) time of not less than 4 hours was required for wheats having the slowest reaction rates. An equation similar to the one used for flour, relating unbound dye concentration to protein content, was developed for whole wheat. Using this equation, another table relating protein content of wheat, unbound dye concentration, and percent transmission can be prepared.

Results

The specific equations for calculating the protein content of the wheat or flour samples as a function of the amount of dye bound or unbound are described in this section.

Estimation of Protein in Flour. Figure 1 shows a plot of the dye

[5] A description of this cell has been presented to *Analytical Chemistry* for publication. Briefly, it consists of two pyrex plates sealed together so that the space between them gives a total light path of only 0.2 mm. A partial vacuum serves to draw the samples through the cell. It was designed to fit in the regular tube holder.

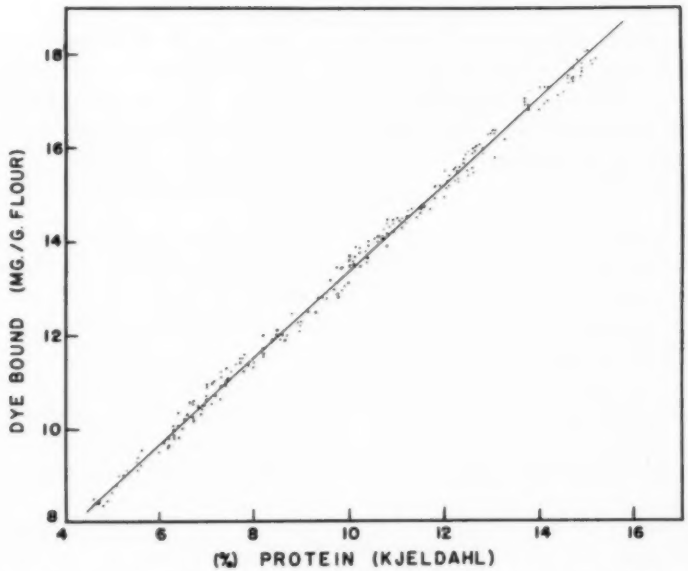


Fig. 1. Regression of percent protein on the mg. of dye bound per g. of flour for 218 different samples. The solid line is a plot of the regression equation. Correlation coefficient is +0.997. Standard error of estimate is 0.20%.

bound as a function of the protein content for 218 samples of flour. Experience has shown that the amount of dye bound by flour is exceptionally constant for samples from a given source. The only difference found could be attributed to experimental error. This error should not be more than $\pm 1\%$ of the apparent protein content.

The regression equation for the correlation of dye bound with protein content is:

$$B = 0.915P + 4.23 \qquad (1)$$

where B is mg. of dye bound per g. of sample, and P is percent protein. This plot gave a correlation coefficient of +0.997, and the plotted points had a standard deviation from regression of 0.20 protein percentage units. Since the intercept is not zero, it is apparent that constituents other than protein are also binding some of the dye. This will be discussed later.

The mg. of dye bound per g. of sample were calculated from the following relationship, which gives the difference between the dye added and the dye unbound, divided by the weight of the sample:

$$B = (VC_o - VC)/W \qquad (2)$$

where V is ml. of stock dye added, C_0 is concentration of stock dye in mg. per ml., C is concentration of unbound dye at equilibrium, and W is weight of sample in g.

By substituting equation (2) in equation (1) and putting in the actual quantities used, the following relationship, which equates P in terms of C, is obtained:

$$P = 40.92 - 45.54C \quad (3)$$

A table which relates P, C, and % transmission may be prepared using this equation and a plot which gives the relation between known dye concentrations and percent transmission.

Estimation of Protein in Ground Whole Wheat. The same general steps, as outlined above for flour, were taken in evaluating the regression equation for 128 samples of ground whole wheat. A plot showing the correlation of B with P (macro-Kjeldahl) is presented in Fig. 2. The standard error of estimate is 0.22 protein percentage units, and the correlation coefficient is +0.992. The regression equation for the data is:

$$B = 1.00P + 5.53 \quad (4)$$

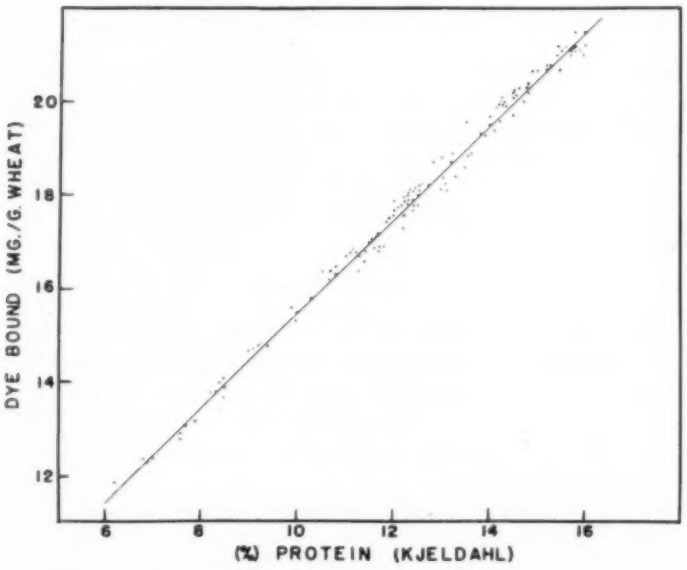


Fig. 2. Regression of percent protein on the mg. of dye bound per g. of ground whole wheat for 128 different samples. The solid line is a plot of the regression equation. Correlation coefficient is +0.992. Standard error of estimate is 0.22%.

A working equation for the calculation of protein in ground whole wheat can be obtained in a manner analogous to that used for flour. After carrying through these rearrangements and substitutions, it is found that:

$$P = 44.47 - 50.00C \qquad (5)$$

From this equation one can construct another table which relates the protein content of whole wheat with the concentration of unbound dye at equilibrium.

A summary of some of the facts cited above plus other incidental information is tabulated below.

	Wheat (128 samples)	*Flour* (218 samples)
Protein content, %		
Minimum	6.2	4.6
Maximum	16.0	15.2
Mean	12.4	10.0
Standard deviation	2.6	2.7
Regression equation: B =	1.00 P + 5.53	0.915 + 4.23
Standard error of estimate, %	0.22	0.20

Absorption Band of Orange G. The broad absorption band of orange G in the region of 485mμ is illustrated in Fig. 3. Solutions of the dye are exceptionally stable, and color intensities followed Beer's Law under the conditions specified above. A close check must be kept on the absorbance of the standard reference or stock solution, since

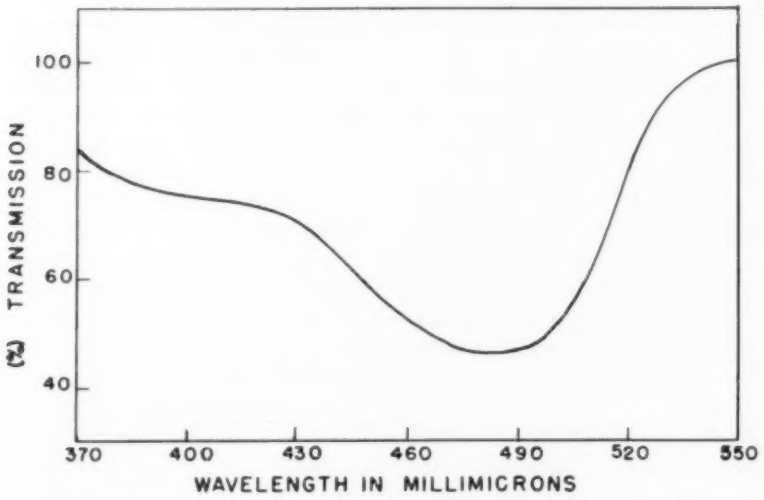


Fig. 3. Absorption curve of an aqueous orange G dye solution using a Bausch & Lomb "Spectronic 20" colorimeter.

every reading is referred to this value. Normally this value is quite constant, though a change in the quality of the transmitted light will alter it, and a new relationship between optical density and dye concentration must then be determined.

Because of the small differences in concentration which are being measured and the need of estimating the transmission to 0.1%, the stability of the colorimeter, and the attention required in this step of the method are of utmost importance. Readings would be easier to make if the galvanometer scale were longer.

Discussion

The regression equation found for straight-grade flour may not hold for various other *grades* of flour, since it is quite clear from the results presented that whole wheat must be considered as an entirely separate material from that of straight-grade flour. Durum types were not included in the samples examined in this study.

Preliminary experiments indicated that the amount of dye bound by the protein in flour was very nearly equal to the difference between the total dye bound and that bound by starch. This fact was demonstrated from the known protein content of a flour sample and from a binding capacity value calculated for the total protein in flour. This total protein-binding value was based on the constant binding capacities and amounts of the individual protein fractions as recorded in a previous communication (7).

Starch binds approximately 4.6 mg. of dye per g. at pH 2.2. Although the starch content was not accurately known for a given flour sample, an average value of 67% gave consistent results. This percentage of starch in flour is reasonable when compared with values published in the literature (1). A 10% fluctuation in this value is equivalent to less than 0.2 mg. of bound dye, and the amount of dye bound by the protein when calculated as the difference between total dye bound and that bound by starch is still within the error of estimate.

Later experiments showed that the bran also bound significant amounts of dye. Furthermore, the binding and/or absorption capacities of both starch and bran varied with the concentration of unbound dye. If it were practical to control this latter quantity, it is conceivable that the protein content of flour could be directly calculated from binding capacity data alone. This is untenable, however, because it would require a foreknowledge of the approximate protein content of the sample.

Although a reaction time of 4 hours was required for wheats having the slowest reaction rates, the majority of wheat samples re-

quired less time than this. The time necessary probably is related to the bran thickness and exposed surface area, as well as the manner of shaking. Accordingly, some wheat samples were reduced in a Weber Pulverizing Mill employing the smallest screen (perforations of 0.024-in. diameter). The maximum reaction time required was shortened to 45 minutes. A screen of still finer mesh might further shorten the reaction time, although bran reduction would become increasingly more difficult. This mill has not been used routinely, because a satisfactory means of recovering the whole sample easily and rapidly has not been devised.

The excellent correlations obtained for both ground wheat and wheat flour protein by the method outlined here give substantial support to the underlying assumption that the ratios of specific wheat proteins, having definite binding capacities, are nearly constant in different vareties of wheat.

The findings of Pence *et al.* (6) demonstrate that different varieties show variability in the content of given protein fractions. An explanation of this apparent discrepancy probably is the fact that a 3% difference in soluble proteins for two given samples of flour causes a difference in the total protein binding-capacity that is only approximately that of the experimental error. Of the 32 samples tabulated by Pence, the mean difference in albumin plus globulin protein as percent of total protein is only 1.8%.

Considering the basic assumption involved, an extension of this method to other seeds or natural products seems quite feasible. Frey (4) recently has shown that the ratio of alcohol-soluble protein to total protein in oats is constant, irrespective of protein content or variety. On the other hand, he reports that zein is not a constant fraction of the total protein in corn (3). Genetic differences may be a factor here.

Literature Cited

1. Bailey, C. H. The constituents of wheat and wheat products, pp. 161–163. Reinhold: New York (1944).
2. Fraenkel-Conrat, H., and Cooper, Mitzi. The use of dyes for the determination of acid and basic groups in proteins. J. Biol. Chem. **154:** 239–246 (1944).
3. Frey, K. J. The interrelationships of proteins and amino acids in corn. Cereal Chem. **28:** 123–132 (1951).
4. Frey, K. J. The relation between alcohol-soluble and total nitrogen contents of oats. Cereal Chem. **28:** 506–509 (1951).
5. Keys, A. A rapid micro-Kjeldahl method. J. Biol. Chem. **132:** 181–187 (1940).
6. Pence, J. W., Weinstein, N. E., and Mecham, D. K. The albumin and globulin contents of wheat flour and their relationship to protein quality. Cereal Chem. **31:** 303–311 (1954).
7. Udy, D. C. Dye-binding capacities of wheat flour protein fractions. Cereal Chem. **31:** 389–395 (1954).

CONTROLLED DEGRADATION OF WAXY-CORN STARCH BY MALT ALPHA-AMYLASE[1]

R. L. Lohmar, F. B. Weakley, and G. E. Lauterbach[2]

ABSTRACT

Degradations of waxy-corn starch pastes by malt alpha-amylase at 60° were followed viscometrically. The inherent viscosity (0.21–0.44) of the dextrin precipitable by alcohol bore a definite ratio to the relative viscosity of the conversion liquors. An empirical relationship was found between enzyme concentration, specific viscosity of the conversion liquor, and time of conversion. Within limits, it was possible to preselect conditions of conversion which led to desired products. Some of the dextrins are useful as sedimenting agents for red blood cells.

Dextrins produced by action of amylases on starches constitute a class of polysaccharides of widely varying physical properties and of considerable industrial use. In only a few instances, however, have control methods for preparation of alpha-amylase-modified starches been adequately described (6). The limited action (cleavage, usually, of less than 1% of the linkages) of malt alpha-amylase on waxy-corn starch has been studied. Waxy-corn starch consists essentially of amylopectin, the starch material of branched structure. Ordinary starches contain substantial amounts of amylose, the starch material of linear structure. Waxy-corn starch was chosen in order to avoid the complications of heterogeneous substrate. The objective was preparation of dextrins for use in fractionation of human blood. Dr. James L. Tullis of the Blood Characterization and Preservation Laboratory of Harvard University has examined many of these dextrins for their utility in sedimentation of red blood cells. It was found that dextrins prepared under substantially identical conditions of enzyme level, time, and temperature of conversion differed markedly in their inherent viscosities and gave variable results when tested for their sedimenting power.[3] His studies revealed that those dextrins having inherent viscosities of 0.31–0.32 were suitable. The viscosity requirements were far more exacting than ordinarily encountered for modified starches; dextrins of viscosities 0.29 and 0.33 were found to be poor sedimenting agents. Improved control of product properties was attained by operating at lower enzyme levels (enabling more precise control of time), but it was still virtually impossible to obtain dextrins within

[1] Manuscript received October 6, 1955. Contribution from the Northern Utilization Research Branch, Peoria, Illinois, one of the Branches of the Agricultural Research Service, U. S. Department of Agriculture.

[2] Present address: Purdue University, Lafayette, Indiana.

[3] Tullis, J. L., private communication.

the narrow range of suitable viscosities. However, when the conversion was followed viscometrically, it was possible repeatedly to prepare dextrins of reproducible physical properties from any of several lots of starch and with different preparations of alpha-amylase.

Materials and Methods

Two different lots of waxy-corn starch were used for most of the conversions. Starch A was a commercial product. Starch B was separated by conventional methods from variety Iowax 2 by members of the Engineering and Development Section of this laboratory.

Conversions were carried out on nominal 4% pastes (100 g. of starch A, 13.7% moisture, or an equivalent dry weight of starch B, in 2.5 l. of water) at 60°C. and pH 6 in the presence of 0.02 *N* calcium chloride. The desired SKB unitage (5) of alpha-amylase, prepared from barley malt by the Dimler, Bachmann, and Davis (1) modification of the method of Kneen, Sandstedt, and Hollenbeck (2), was added, and the course of conversion was followed viscometrically. Capillary viscometers, similar either to that used by Landis and Redfern (3) for amylase assay or to that used by Wolff *et al.* (9), to follow the acid hydrolysis of dextran, were used to measure flow time of paste at 60°C. Ratio of this flow time to that of water at 60°C. (about 20 seconds for the pipettes used) is referred to, for convenience, as "relative viscosity." The conversion was terminated by lowering the pH of the conversion liquors with sulfuric acid. When adjustment to pH 4 was made, relative viscosity ceased to change with time. After 15 minutes, the pH was readjusted to 6. No further change in relative viscosity was noted, indicating irreversible inactivation of the enzyme.

The dextrin was precipitated by an equal volume of 95% ethyl alcohol. The precipitated gum was dissolved in 500 ml. of water, clarified by supercentrifugation, and precipitated in a flocculent form by addition to 5 volumes of alcohol. The product was further dehydrated with alcohol, dried *in vacuo*, and then exposed to the atmosphere at 60% relative humidity to displace adsorbed alcohol.

Several variations of the above procedure were investigated. The routine pasting procedure consisted of gelatinizing the starch on the steam bath, followed by autoclaving at 15 p.s.i.g. for 30 minutes. When autoclaving was omitted, conversion rates were substantially lower. Autoclaving also would tend to give more uniformly dispersed pastes. Autoclaved pastes were used for all preparations reported here, except for the pilot-plant run. Conversions were carried out in both open and closed vessels, but closed vessels were normally used to minimize evaporation.

Products of identical viscosity could be prepared either at 45° or 60°C. However, 60°C. conversion is preferred since activity of any traces of beta-amylase in the enzyme would be minimized at this temperature. Addition of calcium ion had little effect on rate of conversion. It should be pointed out that the alpha-amylase was isolated by a procedure that would supply calcium ions and, therefore, all of these conversions were carried out in the presence of some calcium ion. Added calcium ion did have an effect on stability of alpha-amylase in conversions at 45°C. In the presence of added 0.02 *N* calcium chloride, alpha-amylase was inactivated at pH 3 (15 minutes) but not at pH 3.8. With no added calcium ion, inactivation was found at pH 4. At 60°C., inactivation was shown to occur in either case.

Inactivation was attained alternatively without adjustment of pH, since the alcohol used to precipitate the product also inactivates the alpha-amylase. However, this made precise control of time of hydrolysis difficult and there is some evidence (4) that traces of active alpha-amylase could remain sorbed on the dextrin.

Use of 1.5 volumes rather than one volume of precipitant alcohol did not increase the yield of product, nor did it cause an appreciable change in viscosity of product.

The isolated dextrins were characterized by their reducing power (8) and by their intrinsic and inherent viscosities in water, measured in Ostwald-Cannon-Fenske viscometers (size 50) at 25°C. Inherent viscosities were determined at concentrations such that the ratio of flow time of the solution to that of water was near 1.1. Selected dextrins were further characterized by their fractional precipitability by alcohol (10), by the convertibility by beta-amylase, and by the amount of formic acid produced on oxidation with sodium periodate (4).

Results and Discussion

Determination of viscosity was the most sensitive method of characterizing the precipitated dextrins. When dextrins of the same inherent viscosity were compared, all other analytical values were within the limits of experimental error. For example, two dextrins of nearly identical intrinsic viscosities, 0.304 and 0.307, showed beta-amylase convertibilities of 53 and 51% and gave 0.061 and 0.060 moles formic acid, respectively, per mole of anhydroglucose upon oxidation with sodium periodate. The alcoholic precipitation curves of these two dextrins are shown in Fig. 1.

Oligosaccharides in the supernatant solutions from the precipitation of dextrins had reducing powers corresponding to degrees of polymerization of 15 to 25. Paper chromatography revealed the pres-

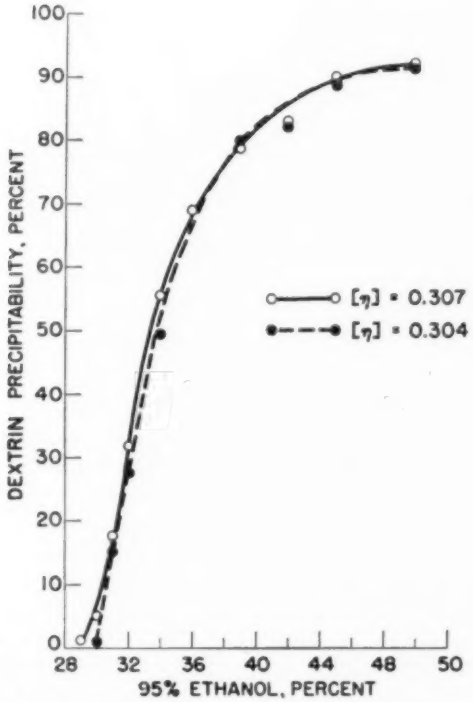


Fig. 1. Alcoholic precipitability of dextrins of similar viscosities.

ence of glucose, maltose, trisaccharide, and higher oligosaccharides.

Starches A and B, and other lots less thoroughly investigated, behaved differently when subjected to the action of alpha-amylase. In Fig. 2 is shown the course of conversion of two lots of starch under identical conditions. Even though one conversion required almost 40% longer to reach the same relative viscosity as the other, the dextrins produced had substantially identical viscosities. It was found that, for all lots of starch investigated, the ratio of inherent viscosity $\{\eta\}$, of the product to relative viscosity of the conversion liquors was near 0.135. This relationship was valid between relative viscosities 1.6 and 3.0, even when the enzyme level was varied sixfold (Table I). Hence, measurement of flow time is an effective control means, even in the absence of precise assay of amylase. Further substantiation of this is given in Table II.

In many technical applications, precipitation would not be used. Experiment showed that viscosity of the unfractionated product is

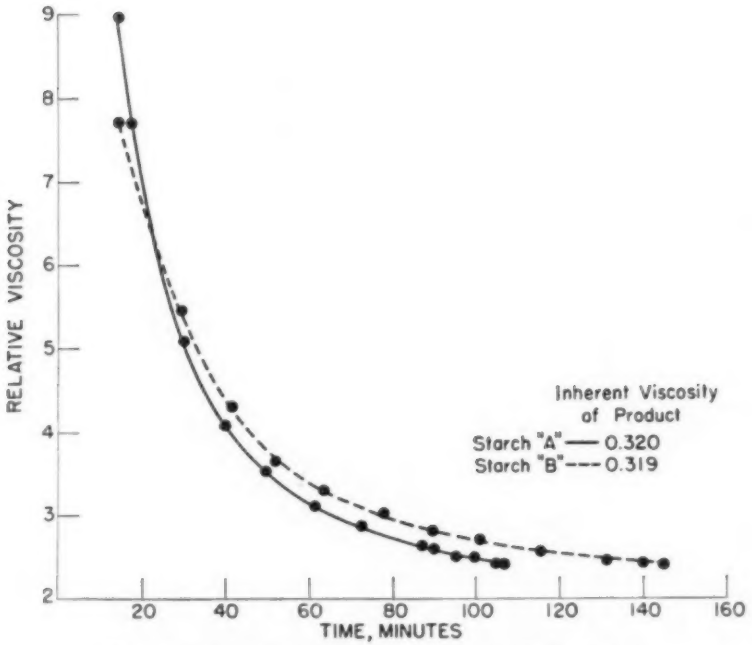


Fig. 2. Conversion of two lots of waxy-corn starch under identical conditions.

TABLE I

ALPHA-AMYLASE DEXTRINIZATION OF WAXY-CORN STARCH — TYPICAL CONVERSIONS

STARCH	CONVERSION				PRODUCT				$\{\eta\}$
	Bonds Cleaved	Amylase units/g. starch	Time	60° η rel	$\{\eta\}$	$[\eta]$	Reducing Power[a]	Yield	η rel.
	%		*min.*					%	
B	0.31	0.036	141	3.78	0.460	0.420	1.1	91	0.122
A	0.33	0.036	150	3.38	0.445	0.438	1.0	88	0.132
A	0.32	0.026	240	2.83	0.370	0.360	1.2	87	0.131
B	0.50	0.218	23	2.63	0.351	0.332	1.1	89	0.135
B	0.50	0.073	147	2.38	0.320[b]	0.315	1.5	87	0.135
B	0.54	0.218	34	2.28	0.309	0.304	1.6	86	0.136
B	0.63	0.218	44	2.07	0.286	0.274	1.7	88	0.138
B	0.85	0.109	150	1.90	0.256	0.248	2.2	81	0.135
B	0.90	0.218	68	1.76	0.234	0.227	2.3	82	0.133
A	1.07	0.218	83	1.63	0.213	0.207	2.9	78	0.131
B	1.40	0.218	150	1.47	0.174	0.172	4.1	83	0.118
B	3.90	0.507	183	1.30	0.132	0.131	6.2	49	0.102

[a] As mg. maltose hydrate equivalent per g.

[b] The unfractionated hydrolysate has $\{\eta\} = 0.289$ and $[\eta] = 0.289$.

TABLE II

REPRODUCTION OF DEXTRINS AT DIFFERENT ENZYME LEVELS

Conversion			Product	
Relative Amylase Level	Time	η rel	$\left\{\begin{matrix}\alpha \\ \beta\end{matrix}\right\}$? $\left\{\begin{matrix}\alpha \\ \beta\end{matrix}\right\}$	Reducing Power[a]
	min.			
3	23	2.63	0.351	1.1
3	28	2.37	0.315	1.8
1	130	2.38	0.318	1.6
3	68	1.76	0.234	2.3
2	150	1.75	0.232	2.8

[a] As mg. maltose hydrate equivalent per g.

somewhat below that of the dextrin precipitated by alcohol (Table I, footnote b). A great reduction in viscosity is not expected, because the relatively small amounts of oligosaccharides removed in the supernatant solution have relatively little effect on viscosity, a weight-average function of molecular weight.

Although use of the parameter 0.135 allowed the operator to inactivate amylase at the desired point in the conversion, there was still a need for a method to permit preselection of conditions such that the desired viscosity would be reached in a convenient time. It is evident that viscosity of conversion liquors is related in some inverse fashion to both enzyme level and time. No mathematical expression applicable to a number of starch samples was found for these relationships. However, if attention was limited to one lot, B, of waxy-corn starch and to amylase levels between 0.036 and 0.22 SKB units per g. of starch, it was found that the following equation described the progress of conversion:

$$(\eta\text{rel}^{-1})\ E \times t = 0.049t + 7.75$$

ηrel = relative flow time (60°C.) of conversion liquors;
E = SKB units of enzyme per gram of starch; and
t = time in minutes.

Times ranged from 28 to 150 minutes. Some examples of predicted and found times are given in Table III. It is seen that good agreement was found except when a very high amylase level was used. It is probable that similar equations could be found for other lots of starch and enzyme levels if more conversion data were obtained.

It is known that there is an alpha-amylase conversion limit for a given amylase level. This is reflected in the above equation, for when $(\eta\text{rel}^{-1})\ E = 0.049$, t becomes infinite. A limitation is also illustrated

TABLE III

VARIATION OF CONVERSION TIME WITH AMYLASE LEVEL

Amylase SKB units/ g. starch	η rel^{-1}	Time	
		Calculated	Found
		min.	*min.*
0.036	2.78	152	141
0.073	1.42	142	133
0.218	1.37	31	28
	0.76	66	68
	0.47	144	150
0.507	0.30	75	183

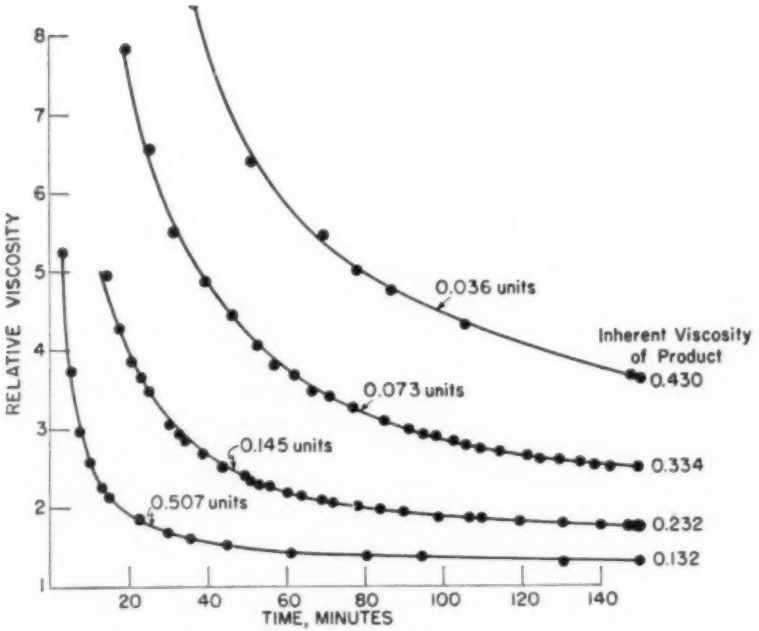


Fig. 3. Conversion of starch at different enzyme levels.

in Fig. 3, where hydrolysis of starch at four different enzyme levels is shown. Characteristics of an "alpha-amylase limit dextrin" are functions of the source of enzyme and of the conversion conditions and are not determined solely by structure of the substrate, as are those of beta-amylase limit dextrins, which represent true limits of conversion.

Pilot-Plant Conversions. Conversions have been successfully carried

through a 45-fold increase in scale. Operating conditions were the same as in the laboratory except that the paste was prepared by heating the starch-water slurry to 200–210°F., without autoclaving. Data obtained on a 100-g. laboratory run and a 10-lb. pilot-plant run are given in the table below. The only notable difference is that the pilot-plant run took 50% longer time to reach the desired relative viscosity. In addition to differences introduced by autoclaving of the laboratory

Comparison of Laboratory and Pilot-Plant Conversions

Conversion		
Weight of starch	100 g.	10 lb.
Relative amylase level	1	1
Time, minutes	130	195
Relative viscosity	2.38	2.35
Product		
Yield, %	85	85
Inherent viscosity	0.318	0.315
Maltose hydrate equivalent, mg/g	1.6	2.2

pastes, there apparently are other factors since, in general, times of hydrolysis were more variable in the larger-scale stainless-steel equipment than in the glass flasks used in the laboratory. This may be caused by differences in agitation, particularly during pasting of starch prior to addition of enzyme. In addition to data in Table IV, it was found that the two dextrins were each effective in sedimentation of red blood cells (see footnote 3) and showed substantially the same fractional precipitability by alcohol.

Acid Conversions. Dextrins having viscosities comparable to those prepared enzymatically were made with the use of 0.1 *N* and 0.2 *N* sulfuric acid as hydrolytic agent. Selected samples varied little from the enzymic dextrins in yield, viscosity, reducing power, or fractional precipitability by alcohol. However, removal of the large amounts of salts from the neutralization of the acid catalyst required that products be reprecipitated several times or be washed repeatedly with 50% alcohol. Furthermore, if any simple relationship existed between relative viscosity of conversion liquors and inherent viscosity of product, it did not cover as wide a range as with the enzyme conversions. This is probably a reflection of the more specific type of hydrolysis by alpha-amylase (4). Thus, although acid hydrolysis can be used to duplicate approximately some of the enzymic dextrins, enzyme conversion is preferred.

Acknowledgment

We wish to thank Mrs. Phyllis Patrick for many of the analyses, Mr. R. A.

Anderson for preparation of waxy-corn starch and for assisting in pilot-plant experiments, and Mrs. Marjorie Austin for a preparation of alpha-amylase.

Literature Cited

1. Dimler, R. J., Bachmann, R. C., and Davis, H. A. Improved techniques for study of the hydrolysis of amylose by malt alpha-amylase. Cereal Chem. **27:** 488–495 (1950).
2. Kneen, E., Sandstedt, R. M., and Hollenbeck, C. M. The differential stability of the malt amylases—separation of the alpha and beta components. Cereal Chem. **20:** 399–423 (1943).
3. Landis, Q., and Redfern, S. Methods for determination of alpha-amylase. III. Improved starch liquefying method. Cereal Chem. **24:** 157–166 (1947).
4. Lohmar, R. L. The structure of α-amylase modified waxy-corn starch. J. Am. Chem. Soc. **76:** 4608–4611 (1954).
5. Olson, W. J., Evans, Ruth, and Dickson, A. D. A modification of the Kneen and Sandstedt methods for the determination of alpha- and beta-amylases in barley malt. Cereal Chem. **21:** 533–539 (1944).
6. Pigman, W. W., Kerr, R. W., and Schink, N. F. U. S. Patent 2,609,326, Sept. 2, 1952.
7. Sandstedt, R. M., Kneen, E., and Blish, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16:** 712–723 (1939).
8. Somogyi, M. A new reagent for the determination of sugars. J. Biol. Chem. **160:** 61–68 (1945).
9. Wolff, I. A., Mehltretter, C. L., Mellies, R. L., Watson, P. R., Hofreiter, B. T., Patrick, P. L., and Rist, C. E. Production of clinical-type dextran. Partial hydrolytic depolymerization and fractionation of the dextran from *Leuconostoc mesenteroides* strain NRRL B-512. Ind. Eng. Chem. **46:** 370–377 (1954).
10. Wolff, I. A., Mellies, R. L., Lohmar, R. L., Hellman, N. N., Rogovin, S. P., Watson, P. R., Sloan, J. W., Hofreiter, B. T., Fisher, B. E., and Rist, C. E. Production of clinical-type dextran. Partial acid hydrolysis and fractionation from *L. mesenteroides* NRRL B-742 and *S. dextranicum* NRRL B-1254. Ind. Eng. Chem. **46:** 2605–2610 (1954).

EFFECT OF CALCIUM STEARYL-2 LACTYLATE IN BREAD MADE WITH NONFAT MILK SOLIDS OF VARYING BAKING QUALITY[1]

W. G. Bechtel, G. E. Hammer, and J. G. Ponte, Jr.

ABSTRACT

The addition of calcium stearyl-2 lactylate to doughs which contained from 3.0% to 8.5% nonfat milk solids of good baking quality resulted in bread of improved quality. Bread of good quality was obtained when the dough was mixed for as long as 2.7 times the optimum mixing time, determined without the use of this additive.

With nonfat milk solids of poor baking quality, use of calcium stearyl-2 lactylate made possible a good loaf of bread with 8.5% nonfat milk solids, provided the dough was given optimum mixing. At lower levels of milk solids (3.0 and 4.0%), mixing time was not so critical.

Farinograph and amylograph tests showed that calcium stearyl-2 lactylate affected the properties of both starch and gluten.

[1] Manuscript received May 9, 1955. The study on which this article is based was made at the American Institute of Baking and was supported by a grant from the C. J. Patterson Company. A report based on the data given herein was presented at the Annual Meeting, St. Louis, Missouri, May 1955.

Thompson and Buddemeyer (6) have reported that the use of calcium stearyl-2 lactylate increased the mixing tolerance of doughs without materially altering the optimum mixing time. They reported also that this additive improved loaf volumes and quality scores of bread when the doughs were mixed for various times from 1 minute to 13 minutes.

This paper is based on an investigation of the effect of calcium stearyl-2 lactylate upon (a) bread made with varying amounts of nonfat dry milk solids of both good and poor baking quality, and (b) bread made from mixtures of gluten with wheat starch to give synthetic flours of varying protein content. In addition, a study was made of the effect of calcium stearyl-2 lactylate on starch and gluten.

Materials and Methods

Preparation of Bread. To test the effect of calcium stearyl-2 lactylate in bread which contained different proportions of nonfat dry milk solids, the following sponge-dough procedure was used:

	Sponge	*Dough*
	%	%
Flour	60	40
Water	(a)	(a)
Yeast	2.0	
Yeast food—Arkady	0.5	
Malt flour, 20° L.	0.5	
Salt		2.0
Sugar—sucrose		4.0
Nonfat dry milk solids		(b)
Lard		2.0
Calcium stearyl-2 lactylate		0.5

a. As determined by the farinograph and a preliminary baking test.
b. In different doughs 3.0%, 4.0%, 6.0% and 8.5%, based on the flour, were used.

Mixing: Hobart A-120 mixer, McDuffee bowl and fork. Sponges were mixed 1 minute at speed 1 and 1 minute at speed 2. Doughs were mixed 1 minute at speed 1, followed by 1.5 minutes, 3 minutes, 5 minutes, or 8 minutes at speed 2, as required by the experiment.

Fermentation: Sponge temperature after mixing was 75°F. to 76°F. (24°C. to 24.5°C.). Sponge fermentation time was 4 hours at 85°F. (29.5°C.) and 85% relative humidity. Dough temperature after mixing, 80°F. to 81°F. (26.7°C. to 27.2°C.). Floor time 20 minutes. Intermediate proof 12 minutes. Scaled 18.5 oz. Pan proofed at 98°F. to 100°F. (36.6°C. to 37.8°C.) to uniform height. Baked 23 minutes at 450°F. (232°C.).

Bread made from wheat starch and gluten instead of flour was made by the technique of Bechtel and Meisner (2). Gluten, washed from flour and kept frozen without drying, and wheat starch were used in the proportions required to give "flour" of 8%, 10%, and 12% protein at 14% moisture.

Specific Volume and Score of Bread. The loaves were allowed to cool on racks for 2 hours. They were then wrapped and sealed in waxed paper. The internal temperature of the loaves at time of wrapping was 95°F. (35°C.). After 24 hours the loaves were weighed, the volumes were determined by displacement of rape seed, and the bread was scored on the following basis:

External	Perfect Score	*Internal*	Perfect Score
Volume (a)	10	Grain	10
Color of crust	8	Color of crumb	10
Symmetry of form	3	Aroma	10
Evenness of bake	3	Taste	15
Character of crust	3	Mastication	10
Break and shred	3	Texture	15
Total	30	Total	70

a. Bread of specific volume between 9.5 and 10.4 cu. in. per oz. was given the score 10. For each unit above 10.4 or below 9.5, the score was reduced by one-half point.

As scored by expert bakers, 80 was considered the minimum quality for a salable loaf. The score 80 to 82 was a fair loaf, 83 to 85 was a good loaf, and 86 to 90 a very good loaf.

Laboratory Determinations. Amylograph consistency curves were obtained, using 50 g. of wheat starch and 450 ml. of distilled water buffered to pH 5.3. Calcium stearyl-2 lactylate was mixed with the dry starch. Farinograph curves were made using 160 g. of gluten washed from flour. This product contained 65% water, and the protein content, on the dry basis, was 70%. The gluten was at 30°C. at the start of the test. Calcium stearyl-2 lactylate was sifted onto the gluten during the first few seconds of mixing.

Results and Discussion

Nonfat Milk Solids of Good Baking Quality. Two samples of roller-dried and one of spray-dried nonfat milk solids of good baking quality were used at 3.0, 4.0, 6.0 and 8.5%, based on the flour. The results with these three samples were in good agreement, and have been averaged to obtain the values given for specific volume and quality score in Table I. While the specific volume of bread which contained calcium stearyl-2 lactylate was somewhat higher, with an average difference in excess of 0.1 cu. in. per oz., this difference was not significant

at the 5% level. There was a considerable difference in the quality scores. For equal milk content and the same dough mixing time, bread which contained calcium stearyl-2 lactylate invariably received the higher score. It had been established that the optimum mixing time for the winter wheat flour employed was 3 minutes. It is apparent from Table I that bread quality was improved at all mixing times and at all levels of milk employed. This confirms and extends the findings of Thompson and Buddemeyer (6), who made a mixing time study using nonfat dry milk solids at 3%, based on the flour.

Nonfat Milk Solids of Poor Baking Quality. Calcium stearyl-2 lactylate also improved the quality of bread made with nonfat dry milk solids of poor baking quality. This is shown in Table II, in which the data are averages of two experiments which were in good agreement. In one a roller-dried milk was used and in the other, spray-dried. The specific volume of loaves which contained calcium stearyl-2 lactylate was, on the average, 0.2 cu. in. per oz. greater than that of the control loaves. This difference was not significant at the 5% level. The quality scores show that addition of calcium stearyl-2 lactylate made possible a good loaf of bread with as much as 8.5% of dry milk solids of poor

TABLE I

EFFECT OF 0.5% CALCIUM STEARYL-2 LACTYLATE ON SPECIFIC VOLUME AND QUALITY SCORE OF BREAD MADE WITH NONFAT MILK SOLIDS OF GOOD BAKING QUALITY

Nonfat Milk Solids	Mixing Time	Specific Volume		Quality Score	
		Calcium Stearyl-2 Lactylate	Control	Calcium Stearyl-2 Lactylate	Control
%	*min.*	*cu. in./oz.*	*cu. in./oz.*	*Score*	*Score*
3.0	1.5	8.1	8.1	77	73
	3.0	10.0	9.5	85	81
	5.0	9.8	9.6	87	82
	8.0	9.7	9.4	84	81
4.0	1.5	8.3	8.2	75	73
	3.0	9.8	9.7	86	83
	5.0	9.7	9.7	86	83
	8.0	9.5	9.6	83	80
6.0	1.5	8.1	8.1	74	70
	3.0	9.6	9.5	87	83
	5.0	9.8	9.5	86	83
	8.0	9.2	9.3	84	80
8.5	1.5	8.6	8.5	83	78
	3.0	10.1	9.9	86	82
	5.0	9.9	9.9	86	82
	8.0	9.7	9.3	84	79

TABLE II

EFFECT OF 0.5% CALCIUM STEARYL-2 LACTYLATE ON SPECIFIC VOLUME AND QUALITY SCORE OF BREAD MADE WITH NONFAT MILK SOLIDS OF POOR BAKING QUALITY

Nonfat Milk Solids	Mixing Time	Specific Volume		Quality Score	
		Calcium Stearyl-2 Lactylate	Control	Calcium Stearyl-2 Lactylate	Control
%	*min.*	*cu. in./oz.*	*cu. in./oz.*	*Score*	*Score*
3.0	1.5	8.2	7.9	73	70
	3.0	9.7	9.5	87	81
	5.0	9.6	9.5	85	82
	8.0	9.2	9.1	82	79
4.0	1.5	7.9	8.0	73	71
	3.0	9.3	9.1	85	84
	5.0	9.4	9.4	83	82
	8.0	9.2	8.8	80	77
6.0	1.5	7.8	7.7	75	76
	3.0	9.3	9.3	86	84
	5.0	9.2	9.0	84	82
	8.0	8.6	8.2	79	75
8.5	1.5	7.9	7.7	72	70
	3.0	9.1	8.9	83	78
	5.0	9.4	9.0	82	78
	8.0	8.9	8.4	75	73

baking quality, provided the dough was given optimum mixing. At lower levels of milk, mixing time was not so critical, as shown by the comparatively high quality scores of the bread from overmixed doughs.

The quality attributes which were improved by calcium stearyl-2 lactylate were principally internal, although break and shred were also improved. A finer, more uniform grain was obtained. The texture was smoother and the crumb lighter in color. It was observed that the crumb and crust were more tender.

Bread Made from Starch and Gluten. Bread was made from the

TABLE III

EFFECT OF 0.5% CALCIUM STEARYL-2 LACTYLATE ON SPECIFIC VOLUME AND QUALITY SCORE OF BREAD MADE WITH STARCH AND GLUTEN INSTEAD OF FLOUR

Protein in Starch-Gluten Mixture	Specific Volume		Quality Score	
	Calcium Stearyl-2 Lactylate	Control	Calcium Stearyl-2 Lactylate	Control
%	*cu. in./oz.*	*cu. in./oz.*	*Score*	*Score*
8	8.8	8.0	79	74
10	8.8	8.4	83	79
12	9.0	8.4	86	81

flour fractions gluten and starch obtained from a spring-wheat patent flour. In this manner the effect of calcium stearyl-2 lactylate on loaf volume and quality score could be studied over a wide range of flour-protein values while using the same components. If bread had been made by use of different flours, protein quality as well as quantity might have varied. Calcium stearyl-2 lactylate improved the bread greatly when the protein in the gluten and starch totaled 8, 10, or 12%. Data for specific volumes and quality scores in Table III suggest the possibility that calcium stearyl-2 lactylate might be especially advantageous in improving bread made from flours of relatively low protein content.

Effect of Calcium Stearyl-2 Lactylate on Starch Gelatinization. Figure 1 shows that the effect of this compound was to retard the gelatinization and swelling of the starch. In the control test the consistency curve began to rise at 80°C., and reached a plateau at 600 Brabender Amylograph Units after 5 minutes at 92°C. In the test with calcium stearyl-2 lactylate the consistency curve began to rise at 90°C., and after 5 minutes at 92° the consistency was only 270 Brabender Units. The curve continued to rise, although at a slower rate, for 30 minutes at 92°, after which there was a rapid increase in consistency to and above 1,000 Brabender Units.

These results suggest that a function of calcium stearyl-2 lactylate in breadmaking is to retard the swelling of starch during baking. Microscopic studies by Baker (1), Sandstedt, Schaumburg, and Fleming (5), and by others have shown that bread-crumb structure consists of a continuous protein film in which the swollen starch granules are em-

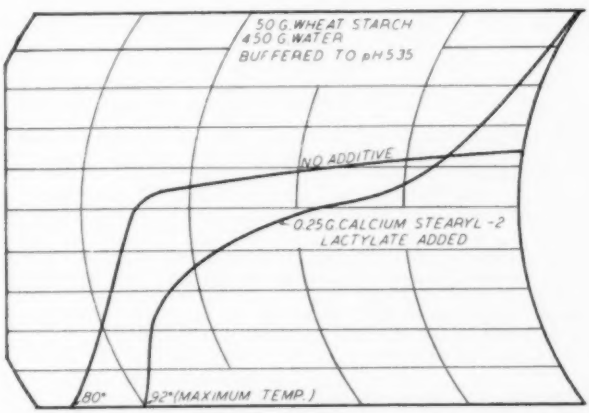


Fig. 1. Amylograph curves showing the effect of calcium stearyl-2 lactylate on the gelatinization of wheat starch.

bedded. The granules are separated from one another by layers of protein. Prentice, Cuendet, and Geddes (4) pointed out that increasing the protein content of flour would tend to decrease the association of the starch granules in such films, and that this should decrease the rate of firming of the bread. In a similar way, if the gelatinization and swelling of starch were retarded during baking, the ratio of volume of starch to that of protein would be lessened, and the bread should have a less firm structure. It was found by Bechtel and Meisner (3) that crumb texture appears to be a property of gluten, and that with increased gluten content the texture becomes smoother and less harsh. We might expect that decreased swelling of the starch granules would likewise give increased smoothness of texture to bread.

Effect of Calcium Stearyl-2 Lactylate on Gluten. When gluten, washed from flour but not dried, was mixed in the farinograph the mixing curve was very irregular, with sudden large dips. It was observed that the gluten formed cylindrical cores about the mixing blades and that the dips were due to slippage of these gluten cylinders past each other. When 1 g. of calcium stearyl-2 lactylate was added to a fresh sample of 160 g. of the wet gluten, the mixing curve was relatively regular. In a 10-minute mixing period the greatest sudden irregularity in the curve was 30 Brabender Farinograph Units in contrast to the control in which the dips were up to 150 units. In this experiment the gluten appeared somewhat crumbly, especially in the first few minutes of mixing. It mixed readily without forming cylinders around the blades. From this it is concluded that calcium stearyl-2 lactylate alters the structure of the gluten. The possibility was suggested by Thompson and Buddemeyer (6, 7) that this is caused by a colloidal binding of the anion to the flour protein.

Literature Cited

1. Baker, J. C. The structure of the gas cell in bread dough. Cereal Chem. **18:** 34–41 (1941).
2. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. I. Fractionation of flour and preparation of bread. Cereal Chem. **31:** 163–170 (1954).
3. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. IV. Effect of gluten and wheat starch. Cereal Chem. **31:** 182–187 (1954).
4. Prentice, N., Cuendet, L. S., and Geddes, W. F. Studies on bread staling. V. Effect of flour fractions and various starches on the firming of bread crumb. Cereal Chem. **31:** 188–206 (1954).
5. Sandstedt, R. M., Schaumburg, Lorene, and Fleming, J. The microscopic structure of bread and dough. Cereal Chem. **31:** 43–49 (1954).
6. Thompson, J. B., and Buddemeyer, B. D. Improvement of flour mixing characteristics by a stearyl lactylic acid salt. Cereal Chem. **31:** 296–302 (1954).
7. Thompson, J. B., and Buddemeyer, B. D. A comparison of the effects of bromate and calcium stearyl-2 lactylate on bread quality. Cereal Chem. **32:** 493–498 (1955).

THE EFFECT OF LIGHT ON VITAMIN RETENTION IN ENRICHED WHITE BREAD[1]

KENNETH MORGAREIDGE[2]

ABSTRACT

The retention of vitamin content in enriched bread was studied under practical retail conditions, to determine the effect of transparent vs. semi-opaque wrapping materials. Plain cellophane, printed cellophane, and printed waxed paper were compared at three levels of illumination. The results show that, regardless of the type of wrapper, there was no loss of thiamine, riboflavin, or niacin when bread displays were subjected to normal intermittent illumination for periods up to 5 days.

The present investigation was undertaken to determine whether enriched white bread suffers significant losses in vitamin content as a result of normal exposure to light such as may be encountered during distribution and display in retail channels. Of the three vitamins regularly added to bread, only riboflavin is known to be easily degraded by photochemical reactions under certain conditions. For the sake of completeness, this study also included thiamine and niacin.

The modern trend toward higher levels of illumination in retail stores and toward greater use of transparent film wrappers on bread made it desirable to compare the two most widely used wrapping materials, cellophane and waxed paper. Loy, Haggerty, and Combs (3), in emphasizing the importance of protecting enriched bakery products from undue exposure to light during preparation of analytical samples, observed that cellophane appeared to offer very little protection for riboflavin in partially baked rolls, whereas heavy waxed paper gave some measure of protection to ordinary "pan" bread. These workers did not make a direct comparison between the two types of wrappers, and the conditions of exposure used were somewhat severe in comparison with normal indoor illumination. In view of this, it appeared of interest to design an experiment in which the conditions of exposure would be realistic, both in regard to intensity of light and to the usual manner in which bread is displayed.

In order to provide a factual basis for the choice of illumination levels, information was sought regarding current lighting practice in retail stores where bread is sold. The Illuminating Engineering Society (2) recommends levels of 30 to 70 foot-candles for general mer-

[1] Manuscript received August 29, 1955.

[2] Chief Chemist, Food Research Laboratories, Inc., 48-14 Thirty-Third Street, Long Island City 1, New York.

chandising areas in retail stores. Spot checks were made in the New York City area of the amount of light found in the baked-goods sections of a number of markets. General illumination intensities as high as 70 to 80 foot-candles were found only in the most modern, brightly lighted supermarkets, while many smaller markets and neighborhood stores had but 2 to 10 foot-candles.

More representative data were obtained through a survey made in six eastern states and the District of Columbia in which light-meter readings were taken in 123 stores. Thirty-four of the stores were small neighborhood groceries, 28 were medium-sized markets, and 61 were classified as supermarkets having five or more check-out counters. The average light intensity at a point 5 feet above the floor (eye level) in these three types of stores was 30, 58, and 56 foot-candles, respectively. These readings were taken in the vicinity of the baked-goods displays.

White bread is invariably placed on the lower shelves of typical displays wherein specialty breads and sweet goods tend to be displayed on higher shelves near eye level. Furthermore, white bread was rarely exposed in a single row of loaves but rather was stacked two or three loaves high. Light-meter readings were taken at the front edge of the white-bread shelf in all stores. The average value was found to be 37 foot-candles. As would be expected, there was a wide range of intensities, the lowest being 4 foot-candles and the highest, 138. There was little correlation with the type of store.

In view of the foregoing information, it was decided to provide for three display conditions in the experimental design. Two of the displays were set up in the laboratory under controlled illumination arranged to provide area intensities of 30 and 100 foot-candles, respectively, at eye level. The third display was set up in a modern, well-lighted supermarket at a point adjacent to the store's normal baked-goods section where the area illumination was 80 foot-candles. The general lighting of the store was provided by double lines of fluorescent tubes set in the ceiling. The lighting of the laboratory displays was provided by 40-watt fluorescent tubes arranged in open, white-enameled reflectors suspended 30° off-vertical above and on both sides of open-shelf stands. The height of the reflectors above the floor was then adjusted until meter readings of 30 and 100 foot-candles were obtained at the 5-ft. level midway between them.

The display stands used in the experiment were patterned after a popular commercial design and consisted of three open shelves supported on center posts with 15-in. clearance between them.

In addition to light intensity, the other two variables included

in the experimental design were length of exposure and type of wrapper. Frequent servicing of stores by bakery routemen makes it unusual for bread to be displayed for sale after it is more than 48 to 72 hours old. However, it was decided to extend the period of test observation to 5 days, during which time the lighting would be intermittent in conformity with the normal daily cycle.

Materials and Methods

The bread was obtained from a commercial baker and represented approximately half of a standard dough batch incorporating 600 lbs. of flour. Enrichment was in the form of compressed vitamin tablets which were blended with the yeast suspension before it was added to the sponge mix. To ensure the identity of loaves from a single mix, one sponge was selected and thereafter followed throughout the entire process. At each stage, from the dough divider on, 5-minute breaks in the production lines were introduced to separate the selected batch from the ones immediately preceding and following.

The first 75 loaves in the selected batch which reached the slicing machine were discarded and the next 150 loaves were wrapped in plain cellophane (300-MST-51) with 3-in. paper label band insert and 2½-in. square end-seals.

The next 150 loaves were wrapped in 300-MST cellophane bearing a printed design 5 in. wide which was estimated to obscure about 50% of the exposed area. The paper insert label was omitted but the end labels were applied as in the loaves wrapped in clear cellophane.

The third group of 150 loaves was wrapped in the commercial printed waxed paper in which the brand is regularly sold. This paper was identified as "Mirro-Paque" (Pollock Paper Corp.).[3]

As each group of loaves came from the wrapping machine, they were packed in heavy cardboard cartons (25 loaves per carton) and transported in a closed truck directly to the laboratory, where they remained overnight in the closed cartons. The following morning the bread was distributed into groups for arrangement on the display stands.

In all of the experimental displays, the loaves of bread were stacked three rows high; the bottom row, end-out; the middle row, side-out; and the top row, end-out. Groups of from 10 to 14 loaves were displayed on both bottom and middle shelves in each of the three types of wrappers. The waxed paper and plain cellophane were given the end positions and the printed cellophane the center position in all displays. In the store display, only one side of the stand was

[3] Address: 2236 S. LaMar, Dallas, Texas.

used; in the laboratory displays both sides were used. The lights for the laboratory displays were controlled by a time clock set to provide 13 hours on (8 a.m. to 9 p.m.) and 11 hours off. The lights in the store were on from 7:45 a.m. to 6:15 p.m. four days a week and from 7:45 a.m. to 9:15 p.m. on the other two days.

During the period that bread remains on the retail shelf, seldom more than 48 hours, it is being constantly handled and picked over by customers in self-service outlets. For this study, it was necessary to stock the displays with the entire supply of bread at the beginning of the test period. To give all of the loaves an equal chance of occupying end or top positions during part of the exposure time, the displays were rotated twice (9 a.m. and at 2 p.m.) on each day of the test by removing all the loaves of each wrapper group from the shelves and mixing randomly on the top shelf. They were then restacked on the shelves in the same general arangement as before.

A set of control samples was chosen at random from each group for immediate analysis. Thereafter, at 9 a.m. on each of the succeeding five days, two loaves were selected at random from each wrapper group on each of the three display stands. Thus, six sets of samples were involved, of 18 loaves each, making a total of 108 individual loaves. The individuals were assigned consecutive code numbers and each loaf was analyzed twice for each of the three vitamins. The first and second assays on each loaf were conducted on different days.

The following steps were carried out in preparing the bread samples for analysis. Each loaf was assigned a serial code number according to a predetermined scheme and thenceforth the sample was identifiable by laboratory personnel by code number only. After removal of the wrapper, a tared, stainless-steel rod (ground to a point at one end) was inserted lengthwise through the center of the sliced bread. After the fresh weight was recorded, the individual slices were spread apart on the rod which was then suspended on a rack in a totally dark room maintained at 29.9°–32.2°C. (85°–90°F.) with a gentle forced circulation of air. A numbered tag identified the loaf and was transferred with the sample in each succeeding step of preparation. All operations requiring manipulation of the samples were conducted in darkened areas providing just sufficient light to ensure proper handling.

The system of drying just described permitted essentially complete moisture equilibrium to be reached by the slices in 24 hours, at which time they had lost approximately 35% of their original weight. The dry weight was then recorded, and slices comprising each loaf were

crushed and ground through a Labconco burr mill set at the smallest clearance between the burrs. The finely ground crumbs were thoroughly mixed and filled into 207 by 300 tin cans, after which the lids were sealed on with a hand-operated can-closing machine. The sealed cans were stored at 7.2°C. (45°F.) until needed for assay. At the time the crumbs were filled into the cans, an aliquot was taken for residual moisture determination (air-oven method, 6 hours at 105°C.).

The assay methods employed were those officially adopted by the Association of Official Agricultural Chemists as described in their *Methods of Analysis* (1) and as subsequently modified by official amendment in the *Journal of the A.O.A.C.* through 1955. The values obtained were recalculated to a uniform 62% solids basis according to standard practice in bread analysis.

Results

The 216 values comprising the complete sets of data for each of the three vitamins have been summarized in Table I by averaging the four replicates (two determinations on each of two loaves) for each of the 54 different combinations of wrapper, illumination level, and time of exposure. Table II shows the combined averages for each type of wrapper under each level of illumination over the entire exposure period. Inspection of these averages reveals no evidence of an over-all effect due either to wrapper or light intensity.

The original data were subjected to variance analysis with the results shown in Table III. The mean squares for wrapper and illumination were entirely without significance when tested against the $I \times W$ interaction. The days-of-exposure main effect was found to be significant at the 5% level in the riboflavin data only. On critical examination, the values in Table I show that this was due to a very slight upward trend in the apparent riboflavin content of the samples from day 1 to day 5. This must be attributed to an experimental artifact for which no obvious explanation is apparent. The niacin data exhibit a significant ($p = 0.001$) interaction between illumination level (I) and days of exposure (D) which must also be regarded as an artifact. Table I reveals no general trend but a tendency for low niacin values to occur in the 30-foot-candle laboratory samples on the same days when the supermarket samples were high, and vice versa.

Discussion

It has been demonstrated by the results obtained in this study that normal exposure of enriched white bread to light intensities as high as 100 foot-candles of intermittent general illumination causes

TABLE I

VITAMIN CONTENT OF BREAD[a] UNDER VARIOUS CONDITIONS OF DISPLAY AND ILLUMINATION

VITAMIN AND DAYS EXPOSED	SUPERMARKET (80 Foot-Candles)				LABORATORY (100 Foot-Candles)				LABORATORY (30 Foot-Candles)			
	Cellophane		Waxed Paper	Average	Cellophane		Waxed Paper	Average	Cellophane		Waxed Paper	Average
	Plain	Print			Plain	Print			Plain	Print		
	mg/lb	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*
Thiamine												
0	1.54	1.46	1.47	1.490	1.48	1.55	1.43	1.487	1.47	1.43	1.52	1.473
1	1.43	1.48	1.42	1.443	1.42	1.41	1.53	1.453	1.49	1.44	1.47	1.467
2	1.43	1.46	1.50	1.463	1.48	1.54	1.49	1.503	1.55	1.54	1.49	1.527
3	1.45	1.40	1.44	1.430	1.48	1.46	1.46	1.467	1.52	1.45	1.47	1.480
4	1.46	1.47	1.37	1.433	1.44	1.52	1.55	1.503	1.52	1.55	1.47	1.513
5	1.46	1.46	1.46	1.460	1.49	1.50	1.46	1.483	1.47	1.47	1.49	1.477
Av.	1.462	1.455	1.442		1.464	1.495	1.487		1.503	1.480	1.485	
Riboflavin												
0	1.09	1.10	1.11	1.100	1.16	1.14	1.11	1.137	1.12	1.10	1.09	1.103
1	1.08	1.11	1.08	1.090	1.10	1.09	1.06	1.083	1.06	1.04	1.08	1.060
2	1.12	1.07	1.11	1.100	1.07	1.06	1.06	1.063	1.07	1.07	1.12	1.087
3	1.13	1.11	1.11	1.117	1.08	1.09	1.12	1.097	1.11	1.15	1.07	1.110
4	1.08	1.13	1.13	1.113	1.08	1.10	1.07	1.083	1.09	1.09	1.12	1.100
5	1.10	1.10	1.09	1.097	1.12	1.09	1.16	1.123	1.18	1.11	1.13	1.140
Av.	1.097	1.102	1.101		1.101	1.093	1.095		1.106	1.093	1.101	
Niacin												
0	11.7	10.8	11.4	11.30	11.6	11.2	10.8	11.20	11.6	11.7	12.2	11.50
1	12.5	12.3	12.1	12.30	11.4	11.7	11.5	11.53	10.9	11.0	11.6	11.17
2	11.2	11.0	11.0	11.07	11.8	11.6	11.8	11.73	12.2	12.3	11.8	12.10
3	12.4	12.6	11.3	12.10	11.4	11.3	11.3	11.33	11.3	11.2	11.2	11.23
4	11.6	11.4	11.4	11.47	11.4	11.5	11.5	11.47	11.4	11.6	11.4	11.47
5	11.8	10.8	11.3	11.30	11.8	11.8	11.5	11.70	11.5	11.3	11.6	11.47
Av.	11.85	11.47	11.38		11.55	11.50	11.38		11.46	11.50	11.62	

[a] Values shown are averages of four determinations, two on each of two loaves, calculated to uniform 62% solids basis.

TABLE II

AVERAGE VITAMIN CONTENT OF BREAD SAMPLES

VITAMIN	WRAPPER	ILLUMINATION			AVERAGE
		100 Foot-Candles (Laboratory)	80 Foot-Candles (Supermarket)	30 Foot-Candles (Laboratory)	
		mg/lb	*mg/lb*	*mg/lb*	*mg/lb*
Thiamine	Plain cellophane	1.464	1.462	1.503	1.476
	Printed cellophane	1.495	1.455	1.480	1.477
	Waxed paper	1.487	1.442	1.485	1.471
	Average	1.453	1.482	1.489	....
Riboflavin	Plain cellophane	1.101	1.097	1.106	1.101
	Printed cellophane	1.093	1.102	1.093	1.096
	Waxed paper	1.095	1.101	1.101	1.099
	Average	1.096	1.100	1.100	....
Niacin	Plain cellophane	11.55	11.85	11.46	11.62
	Printed cellophane	11.50	11.47	11.50	11.49
	Waxed paper	11.38	11.38	11.62	11.46
	Average	11.48	11.56	11.53	....

TABLE III

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARES		
		Thiamine	Riboflavin	Niacin
Total	215			
Between analyses within loaves	108	0.00304	0.00282	0.38065
Between loaves	54	.00408	.00252	0.20796
Illumination (I)	2	.02629	.00062	0.13420
Wrappers (W)	2	.00060	.00030	0.50505
I × W (error)	4	.00569	.00499	0.64768
Days exposed (D)	5	.01066	.00945*	0.28086
I × D	10	.00451	.00457	2.37188***
W × D	10	.00393	.00227	0.25488
I × W × D (error)	20	0.00772	0.00275	0.34868

no detectable reduction in riboflavin content within 5 days. This is true for bread wrapped in cellophane and in waxed paper. The same conclusion applies to thiamine and niacin.

Several factors are probably responsible for the negative findings, especially as regards riboflavin. Although an area illumination of 100 foot-candles represents close to the maximum level likely to be encountered in commercial displays, the universal custom of displaying bread on lower shelves results in a much lower incident intensity on

the exposed portions of the loaves themselves. Furthermore, bread is usually partially shaded by both adjacent loaves and by higher shelves. The experimental conditions imposed on the design of this study corresponded in these respects to general commercial practice.

In commenting on the complete lack of significance of variation between riboflavin retention in loaves wrapped in a transparent film and those wrapped in translucent waxed paper, it may be mentioned that the normal dark crust of average white bread probably affords sufficient protection for the vitamin in all but the most extreme conditions of light exposure.

It is concluded that under ordinary conditions of display, the vitamins present in enriched white bread are not affected by the type of wrapper or by length of exposure wthin the normal shelf-life of the product.

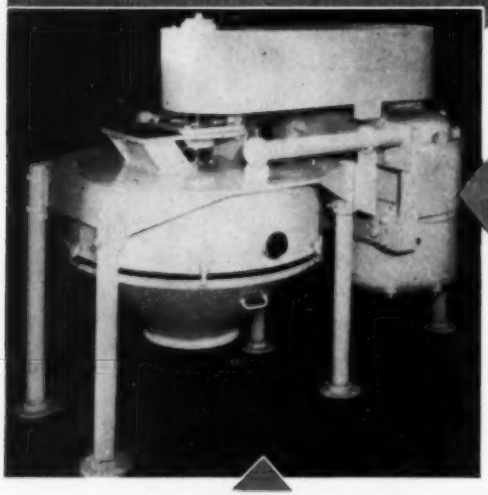
Acknowledgments

The study here reported was sponsored by E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware. The cooperation of Mr. Frank Fischer of the Fischer Baking Company, Newark, New Jersey, in making available the plant facilities necessary for the production and wrapping of the bread used is gratefully acknowledged. The survey of lighting conditions in retail stores was conducted by Mr. Fred C. Clarke of the du Pont Company, whose aid was of much value. We are indebted to Dr. Lila K. Randolph for statistical analysis of the data. The assistance of Mr. Bernard M. Blank of Food Research Laboratories in the technical aspects of this study is deeply appreciated.

Literature Cited

1. Association of Official Agricultural Chemists. Official methods of analysis (7th ed.). The Association: Washington, D. C. (1950).
2. Illuminating Engineering Society. I. E. S. lighting handbook (2nd ed.), pp. 9–67. The Society: New York (1954).
3. Loy, H. W., Haggerty, J. F., and Combs, E. L. Light destruction of riboflavin in bakery products. Food Research **16**: 360–364 (1951).

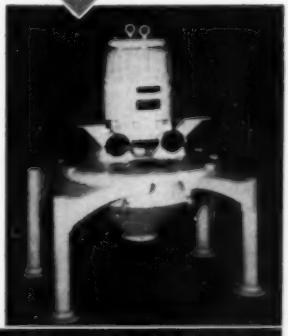
500 CWT PER HOUR!!!
it's a cinch with the NEW "BIG INCH"....



for Positive Infestation Control . . .

the NEW "ENTOLETER" 'BIG INCH' for 150 to 500 cwt per hour

the STANDARD "ENTOLETER" unit for 25 to 150 CWT per hour



You guarantee your flour specifications... let "ENTOLETER" Infestation Control assure the complete cleanliness of all ingredients and finished products . . .

. . . the trademark "ENTOLETER" on milling equipment is your guarantee of complete satisfaction. Write to us for information concerning our products. ENTOLETER DIVISION, The Safety Car Heating and Lighting Company, Inc., 1153 Dixwell Ave., New Haven 4, Connecticut.

The trademark "ENTOLETER" is your guarantee of complete satisfaction

ENTOLETER
CENTRIFUGAL MACHINES

U.S. TIGHTENS MINIMUM SANITARY MILL LIMITS!

BASIS OF LEGAL ACTION
1% OF INSECT DAMAGE

Only 1 Rodent Pellet per Pint Maximum Permitted

The basis of legal action of filth-contaminated wheat will be lowered to the following:

1 or more rodent pellets per pint of wheat (liquid measure) or 1 percent or more of insect-damaged kernels.

The change will be put into effect July 1, 1956, to coincide with the beginning of the crop year, and thus permit time for the Grain Trade to make necessary adjustments.

These levels will be applied in random sampling of cars of wheat.

Excerpt from U.S. Health, Education and Welfare Food and Drug Administration Bulletin M2715.

New Levels Effective During 1956 Crop Year

WASHINGTON, D. C. — The Food and Drug Administration has announced that it will tighten sanitary requirements on wheat beginning July, 1956 to promote increased cleanliness in the Nation's food supply.

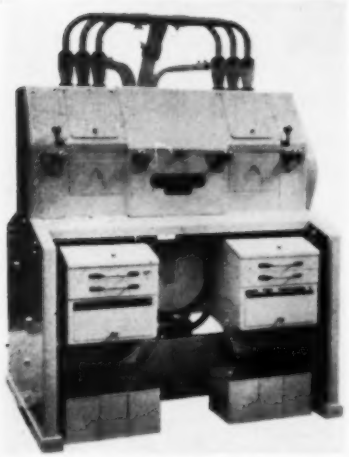
George P. Larrick, Commissioner of Food & Drugs, stated that during the period January through July 31, 1955 wheat from 3754 cars was examined. Of these, 29 cars were found to contain filth in excess of the rodent actionable level, and 3 cars showed insect damage to the extent of 2 per cent or more of the kernels.

The Commissioner said: "Under the Program, substantial progress has been made in improving handling and storage of wheat to prevent contamination. However, experience acquired during the past six months shows that the levels of judgment should be tightened if the program is to continue to be effective."

The New Pneumatic Model MLU 202

BUHLER

AUTOMATIC LABORATORY MILL



Front side of the MLU 202 Buhler Automatic Laboratory Mill; equipped with pneumatic product conveying which eliminates material collecting in the ducts and resultant infestation.

Offers You These Advantages

—It grinds small samples, or large laboratory runs;

—It produces flour with exactly the same baking quality as the flour from a commercial mill;

—It gives results that can be duplicated whenever desired;

—It grinds wheat quickly and automatically; empties completely;

—It is simple to operate and easy to attend;

—It requires no special milling experience from the operator.

The MLU 202 Laboratory Mill was designed primarily to produce flour from any specific sample of wheat so that advance information may be obtained as to what can be expected in yield, ash content, color and baking qualities of the sampled wheat, without running it through a commercial unit. The Buhler MLU 202 has the same advantages as a pneumatic mill in comparison with an elevator mill.

BUHLER BROTHERS, INC. U.S.A.
434 SYNDICATE BLDG.
MINNEAPOLIS 2, MINNESOTA

BUHLER BROTHERS (Canada) LTD.
24 KING ST. WEST
TORONTO 1, ONTARIO

SPROUT, WALDRON & CO., INC.
Buhler Brothers Division
61 LOGAN ST., MUNCY, PENNSYLVANIA

when you use . . .

THE DYOX PROCESS® for flour maturing . . .

The fixed uniformity of DYOX treated flour is a definite advantage to the miller who strives to meet the baker's demand for a starting product of constant value—which works in the bake shops with the least trouble and manipulation by the operator. DYOX treated flour assures overall dependability, uniformity of product, and ease of operation.

"NOVADELOX"® for whiter, brighter flour . . .

"Novadelox" is designed to meet the desire of the consuming public for a bread that is truly white—and to enable millers to produce a uniform product of standard color.

"N-RICHMENT-A"® for uniform enriching . . .

"N-Richment-A" provides a readily available, simple and troublefree product for enriching flour—with the assurance that the standard procedure of adding "N-RICHMENT-A" will produce a dependable standardized flour.

THE N-A FLOUR SERVICE DIVISION with more than a quarter-century of experience . . .

The N-A Flour Service Division with its skilled laboratories and staff is always available to work with you or your consultants on all phases of maturing, bleaching and enriching. Why not phone your nearest N-A Representative today!

DYOX
for flour maturing
NOVADELOX
for a whiter, brighter flour
N-Richment-A
for uniform enrichment

HA-42

NOVADEL FLOUR SERVICE DIVISION

WALLACE & TIERNAN INCORPORATED

BELLEVILLE 9, NEW JERSEY

REPRESENTATIVES IN PRINCIPAL CITIES